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Otago Univ., Dunedin (New Zealand)

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CADMIUM INTAKE VIA OYSTERS AND HEALTH EFFECTS IN NEW ZEALAND

Cadmium Intake, Metabolism and Effects in People with a High Intake of Oysters in New Zealand

by

Joan McKenzie
Department of Nutrition
University of Otago
Dunedin, New Zealand

Tord Kjellstrom
Department of Community Health and General Practice
University of Auckland
Auckland, New Zealand

Ram Sharma
MRC Toxicology Research Unit
University of Otago
Dunedin, New Zealand

Grant No. R807058-01-0

Project Officer

Norman Kowal
Toxicology and Microbiology Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

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16. ABSTRACT

The aim of this study was to confirm the high dietary intakes of cadmium and other trace elements from oysters in a population associated with the oystering industry, and to determine (i) the impact of those high intakes on cadmium concentrations in accessible tissues of the study subjects and (ii) the occurrence of health effects in the population resulting from their dietary exposure to cadmium. Methods for the analysis of cadmium in whole blood, urine and hair by flameless atomic absorption spectrophotometry were established. Seventy-eight subjects participated; from forty-eight of them samples of faeces (3 day), blood, urine (overnight) and hair were obtained both pre-season and end-season (i.e. 6 months later). Questionnaires on oyster intake were administered; dietary and medical questionnaires were administered at the end of the season when height, weight and blood pressure were measured. The faecal output of cadmium confirmed the high intakes; e.g. Category IV subjects were ingesting about 250 µg cadmium/day at the end of the season; calculated intakes for Categories I, II and III were 34 µg/d, 75 µg/d and 116 µg/d, respectively, at the and of the season. The concentration of cadmium in whole blood was higher in the smokers than in the non-smokers. In the non-smokers the increase in whole blood cadmium due to oyster consumption was only 1.2 ng/ml for Category IV. The concentrations of cadmium, zinc and B_2-microglobulin in the urine were unaffected by oyster consumption; there was no indication of glycosuria or proteinuria that could have been attributed to a high intake of cadmium. Hair cadmium, zinc and copper also appeared to be unaffected by oyster consumption. There were no indications of any medical problems that could have been attributed to a high cadmium intake; although the blood pressure of Category III and especially Category IV subjects was relatively higher than that for Categories I and II and they also had a greater body size.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enchance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a co-ordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

The report that follows is the result of co-operative work, and concerns itself with the high cadmium concentration in New Zealand oysters and the high cadmium intake in a small population group with a high oyster intake. The interactions between cadmium and other trace elements in the oysters are of particular interest and it is envisaged these first projects will stimulate further research on the metabolism of cadmium derived from oysters and other foodstuffs.

F. Gordon Hueter, Ph.D
Director
Health Effects Research Laboratory

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PREFACE

During recent years there has been a keen interest among researchers and public health administrators in the toxicity of cadmium, with particular reference to the possible increase of cadmium intake due to increased use of sewage sludge for soil treatment. The risk of an actual increase in the cadmium intake has been well documented and a major remaining question is whether such an increase is likely to lead to any health effects.

So far, only in Japan have population groups with exceedingly high cadmium intakes via food been found. In the high cadmium intake groups in Japan, the prevalence of cadmium-induced proteinuria might be as high as 50% or more. Some studies in Japan demonstrate clear dose-response relationships and these data have been used to analyse the dose-response at relatively low dose levels. Calculations have been supported by metabolic models which involve a number of assumptions, and therefore uniform agreement on the health hazards involved with slight increases of average cadmium intake has not been reached.

Preliminary data from New Zealand indicated that the oysters had such high cadmium levels that a small subgroup of the population who consumed a large number of oysters annually, could have cadmium intakes as high as the affected populations in Japan. The present study endeavoured to document oyster intake in the group with the alleged high oyster consumption, their cadmium intake and also their cadmium concentrations in various biological media such as blood, urine and hair. As the oysters had relatively high levels of selenium, zinc, copper and manganese, these trace elements were also analysed in most of the biological materials collected.

This New Zeland cadmium-exposed group is the only one found outside Japan with such high cadmium intake via food, and it is envisaged that further studies of this group will give valuable data for the explanation of the metabolism as well as the toxic mechanism of cadmium.

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ABSTRACT

The aim of this study was to confirm the high dietary intakes of cadmium and other trace elements from oysters in a population associated with the oystering industry, and to determine (i) the impact of those high intakes on cadmium concentrations in accessible tissues of the study subjects and (ii) the occurrence of health effects in the population resulting from their dietary exposure to cadmium.

Methods for the analysis of cadmium in whole blood, urine and hair by flameless atomic absorption spectrophotometry were established. The analysis of Bluff oysters (Ostrea lutaria) confirmed a high cadmium content (27 µg/g dry wt); the content of other trace elements (zinc, copper, manganese, selenium) was also determined, and differences in the proportions of the trace elements compared with other species of oysters and other shellfish found in New Zealand were observed.

Seventy-eight subjects participated; from forty-eight of them samples of faeces (3-day), blood, urine (overnight) and hair were obtained both pre-season and end-season (i.e. 6 months later). Questionnaires on oyster intake were administered; dietary and medical questionnaires were administered at the end of the season when height, weight and blood pressure were measured. The subjects were classified into four categories of oyster intake with average consumptions being <0.5, 0.5-<2, 2-<6 and ≥6 dozen/week for Categories I, II, III and IV, respectively. The faecal output of cadmium confirmed the high intakes; e.g. Category IV subjects were ingesting about 250 µg cadmium/day at the end of the season; calculated intakes for Categories I, II and III were 34 µg/d, 75 µg/d and 116 µg/d, respectively, at the end of the season. Intakes of zinc were also elevated, and selenium intake would have been doubled for those subjects consuming many oysters.

The concentration of cadmium in whole blood was higher in the smokers than in the non-smokers. In the non-smokers the increase in whole blood cadmium due to oyster consumption was only 1.2 ng/ml for Category IV. Whole blood selenium concentration also was higher in Category IV subjects but their serum zinc and copper concentrations were unaffected. The concentrations of cadmium, zinc and B_2-microglobulin were unaffected by oyster consumption; there was no indication of glycosuria or proteinuria that could have been attributed to a high intake of cadmium. Hair cadmium, zinc and copper also appeared to be unaffected by oyster consumption. There were no indications of any medical problems that could have been attributed to a high cadmium intake; although the blood pressure of Category III and especially Category IV subjects was relatively higher than that for Categories I and II and they also had a greater body size.

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Interactions among the trace elements could have accounted for the only slight effect of cadmium intake from oysters on blood cadmium and for the lack of effect on the other measurements and on the health of the subjects. Further studies would help to elucidate the apparent differences in cadmium metabolism in the subjects in this study compared with other populations exposed to similar high oral intakes.

This report was submitted in fulfillment of Grant No. R807058-01-0 by the University of Otago and the University of Auckland, New Zealand under the sponsorship of the Environmental Protection Agency. This report covers a period from October 1980 to March 1982.

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SECTION I

INTRODUCTION

Cadmium is an element that normally occurs at low concentrations in air, water and food. High occupational exposures may occur, for instance, in the sulphide mineral smelting industry, in cadmium-nickel battery factories, in welding and soldering operations, and in colour-pigment production plants. The number of workers in the United States who have been exposed to cadmium has been estimated at about 100,000 (NIOSH, 1976). Excessive exposure via food has been reported so far only from Japan (Friberg et al., 1974). It may well be that groups in the United States with particular diets have a high dietary cadmium exposure, but the limited studies available have not given any conclusive data. Data from Sweden (Kjellström et al., 1975; Elinder & Kjellström, 1977) indicate that the general environmental cadmium exposure level may have been increasing continuously since the end of the 19th century. Of particular importance here is the possible contribution from sewage sludge (Linnman et al., 1973) and phosphate fertilisers (Stenström & Vahter, 1974; Sharma, 1981) that have been added to soil. Recently, the problem of high cadmium concentrations in sewage sludge in the United States has been discussed (Baker et al., 1979).

It is generally considered (WHO, 1977) that renal tubular damage is the critical effect of cadmium in humans after long term exposures. The dose-response relationship for this effect is not known in detail but epidemiological studies (Kjellström, 1977; Hutton, 1983) indicate that at an average long-term intake of about 200 µg cadmium per day a response rate of 5-20% can be expected. Urinary beta-2-microglobulin excretion, which is a sensitive indicator of cadmium-induced damage (Shiroishi et al., 1977) was used for diagnosis in these studies. Calculations using a metabolic model (Kjellström & Nordberg, 1978) have agreed well with the empirical dose-response relationships and this is the only way, at present, to estimate the daily intake necessary to reach a response rate of, for instance, 1%. Additional epidemiological studies of populations with relatively high daily intakes of cadmium from food are necessary to confirm the estimated dose-response relationships. In order to further develop the metabolic model, additional studies of the relationship between cadmium intake and tissue cadmium concentrations are needed, particularly for cadmium intake via ingestion.

The accumulating reports of an increased incidence of cancer among cadmium-exposed workers in England (Kipling & Waterhouse, 1967), in the United States (Lemen et al., 1976) and in Sweden (Kjellström et al., 1979)

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may make it necessary to re-evaluate the human toxicity of cadmium and emphasize the need for a better understanding of cadmium metabolism.

A previous study of the cadmium content in normal New Zealand diets (duplicate diet method) gave an arithmetic average between 21 and 27 μg/day (Guthrie & Robinson, 1977). The frequency distribution of the cadmium data agreed better with a log-normal distribution than with a normal distribution, however. The mode of the daily intakes was between 10 and 15 μg/day. The two diets with the highest level (118 and 81 μg/day) both contained oysters. In the sample containing 81 μg/day there were two oysters.

A very high cadmium concentration in New Zealand oyster, Ostrea Lutaria (3.31 μg cadmium/g wet weight) has been reported (Guthrie, 1975). A whole range of foods was analysed and in no other sample did the concentration go above 0.3 μg cadmium/g. The reported concentration in cereals and rice agreed well with data from other countries (Friberg et al., 1974). Subsequent analysis of oysters (Nielsen, 1975) has indicated that these very high concentrations (average 5.75 μg Cd/g wet wt.) would thus constitute a high intake source for cadmium.

Fresh or canned oysters are a popular food item in New Zeland (for those who can afford them) and the workers in the canning factories regularly eat many oysters. Preliminary interviews with some of the workers have indicated that the intake might be as high as several dozen oysters per day. The work is seasonal, but continues from about March until September. Nielsen (1975) reported the average weight of the oyster, Ostrea Lutaria, as 8 g. In this study McKenzie (unpublished data) has estimated that the meat in one oyster weighs 7 grams. If the average concentration were 5.75 μg cadmium/g, one dozen oysters per day would contribute about 480 μg cadmium to the daily intake. Even if this consumption continued only for half a year at a time, the risk for cadmium-induced kidney damage after several years exposure must be considerable in view of the experiences from contaminated areas in Japan (Friberg et al., 1974). On the other hand, the high zinc concentration (53 μg/g wet wt., Guthrie, 1975) and the high selenium concentration (0.46 μg/g; Robinson et al., to be published) in the oyster might protect against the cadmium effects in a similar way as animals are protected (Whanger et al., 1980).

Zinc and cadmium are biochemically antagonistic and it has been suggested that the ratios of the two elements can be important in the metabolism of each element (Pier, 1975). Dietary supplements of zinc or copper have been shown to influence the cadmium content in liver, kidney and the jejunal-ileal section of intestinal tissue of young Japanese quail (Jacobs et al., 1977). Zinc generally decreased the cadmium content in the tissues, while copper caused a small increase. The average daily intake of copper and zinc among New Zealand women have been estimated at 1.5 mg and 10 mg respectively (Guthrie & Robinson, 1977). The daily intake of selenium in the Dunedin area ranged between 6 and 70 μg (Griffiths, 1973). In human subjects, and in mice, a greater absorption of cadmium from a test meal has been observed in those with a low body iron store (Flanagan et al., 1978).

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This project covered an initial 18-month period of which the aims were the following:

(a) to develop methods for cadmium analysis in the tissues studied.

(b) to evaluate the cadmium content of various shellfish in New Zealand and the daily cadmium intake in groups with a high shellfish consumption.

(c) to measure the individual and group-average daily cadmium intake of workers in a New Zealand oyster industry.

(d) to measure the interrelationship of cadmium concentrations (and of other trace metals) in serum, blood, urine and hair in the group with a high cadmium intake from oysters and to measure the effect of seasonal variations on the cadmium intake and tissue concentrations.

(e) to measure the occurrence of tubular proteinuria in the group with a high cadmium intake.

It was then envisaged that after the initial project, continued studies would be carried out with particular reference to the interactions between cadmium and other trace elements.

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SECTION 2

CONCLUSIONS

(a) Methods for the estimation of cadmium in blood, urine and hair have been established, and to date appear to be reasonably accurate and reliable. Further inter-laboratory comparisons will be carried out.

(b) Bluff oysters have a high cadmium content; although all species of oysters have a relatively high cadmium content compared to most other foodstuffs, different species show large differences in concentrations of cadmium as well as other trace elements. This is of great interest because a high consumption of different shellfish might reveal differences in cadmium metabolism due to interactions among the elements.

(c) Intakes of cadmium in the population studied were high. Analysis of faeces confirmed the high intakes of cadmium based on analyses of cadmium concentration in oysters and estimates of daily consumption. There was a close agreement between the observed content of cadmium, zinc and copper in faeces and estimated output. Some of the subjects with the highest oyster consumption had daily cadmium intakes greater than 500 µg, which are higher intakes than those shown to be associated with a high incidence of renal tubular damage in people exposed to contaminated rice in Japan. The New Zealand oyster consumers also had intakes of zinc greater than twice the normal New Zealand intake, and intakes of selenium about twice a normal New Zealand intake. The limited data available indicate that the Japanese people with a high cadmium exposure also had intakes of zinc and selenium similar to those of the oyster consumers.

(d) In spite of the very high intake of cadmium from oysters the concentration of cadmium in whole blood was not increased greatly in proportion to the increased intake. The data clearly shows increased blood cadmium concentration due to smoking in the four groups with different levels of oyster consumption. Among non-smokers the increase in blood cadmium due to oyster consumption was only 1.2 ng/ml. There was a good correlation between blood cadmium concentration and oyster intake at the beginning as well as at the end of the oyster season. There were no observed changes during the season or between groups in urinary cadmium output or in hair cadmium concentration. There was a close correlation between blood cadmium and blood selenium concentration, whereas neither serum zinc nor serum copper concentration increased with an increasing blood

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cadmium concentration. There were no differences in urinary life excretion, nor in hair zinc or copper concentration.

(e) The dipstick proteinuria and glycosuria tests did not show any indication of cadmium-induced renal damage. The frequency distribution of urinary beta-2-microglobulin concentration was similar to "control groups" from other epidemiological studies, and none of the participants had a beta-2-microglobulin concentration higher than 250 µg/l (adjusted to specific gravity 1.025).

(f) There was a tendency for higher blood pressures than expected in the groups with the highest oyster intakes. This tendency was influenced by smoking habits but an effect of cadmium on blood pressure could not be ruled out.

(g) Finally, the present study showed that the cadmium in Bluff oysters does not appear to be absorbed to the extent that is considered normal for other foods. The mechanism for this diminished absorption could be interactions with selenium and/or other trace elements, but could also be unusual chemical speciation in oysters compared to other foodstuffs. It could also be that cadmium from oysters is metabolised differently to cadmium from other foods after it has been absorbed.

Further studies on this interesting aspect of cadmium metabolism are possible in New Zealand because different kinds of shellfish with a high cadmium content are freely available, and because great variations in intake of the shellfish exist within the New Zealand population. It is possible that the elucidation of this aspect of cadmium metabolism (i.e. its interactions) could be of great importance for the prevention of cadmium-induced renal damage in other areas of the world with a high cadmium intake.

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SECTION 3

CADMIUM IN OYSTERS AND OTHER SHELLFISH

During the 19891 oyster season 72 Bluff oyster (Ostrea Lutaria) samples were collected for cadmium analysis. These were representative of the oysters consumed by the oystermen in Bluff and by ourselves in the method studies on faeces analysis. The average cadmuim concentration (Table 1) in a composite sample analysed in duplicate was 27 μg Cd/g dry weight. Normally the oyster tissue eaten weighs on average 6-7 g wet weight (giving a cadmium intake of 36-42 μg/oyster), but these were on average larger (average weight 10 g, cadmium intake 70-80 μg/oyster).

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TABLE 1. COMPOSITION OF BLUFF OYSTERS

Constituent	%	μg/g wet wt	μg/g dry wt
Water	79		
Cadmium		5.7	27
Zinc		50.6	241
Copper		4.58	21.8
Manganese		0.73	3.4
Selenium		0.44	2.08

There was also a high cadmium concentration (27 μg Cd/g dry weight) in specimens of paua (Table 2), a shellfish growing on rocks in the same area as where Bluff oysters are harvested. One medium-sized paua had 45 μg Cd/g, but only 11.8 μg Zn/g. Paua have an average total wet weight of 186 g. The results in Table 2 are based on duplicate analysis of a composite sample of 21 pauas.

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TABLE 2. COMPOSITION OF PAUA

Constituent	%	µg/g wet wt	µg/g dry wt
Water	76		
Cadmium		6.5	27.2
Zinc		2.9	12.0
Copper		74	310
Manganese		4.8	19.8
Iron		452	1885

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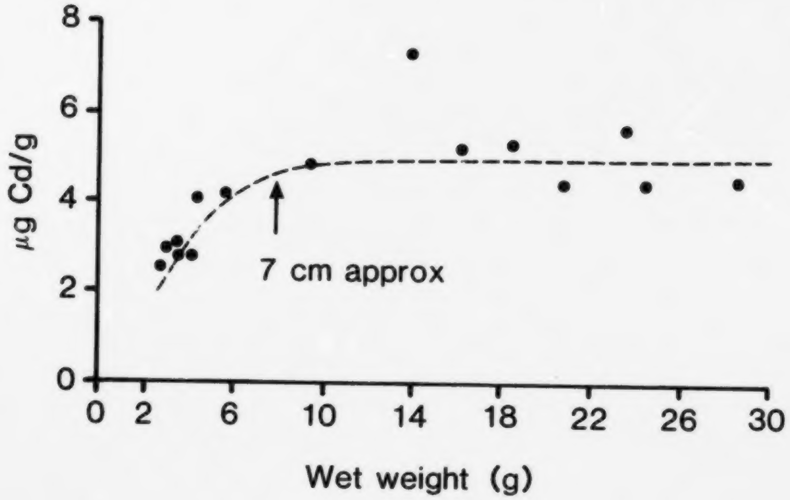

FIGURE 1: CONCENTRATION OF CADMIUM VERSUS BODY WEIGHT FOR OSTREA LUTARIA. (From Nielsen, 1975).

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In an earlier study Nielsen (1975) found that the cadmium concentration increased with size up to about 10 g wet weight, and in larger oysters the concentration ranged between 4-7 µg Cd/g wet wt. (Figure 1).

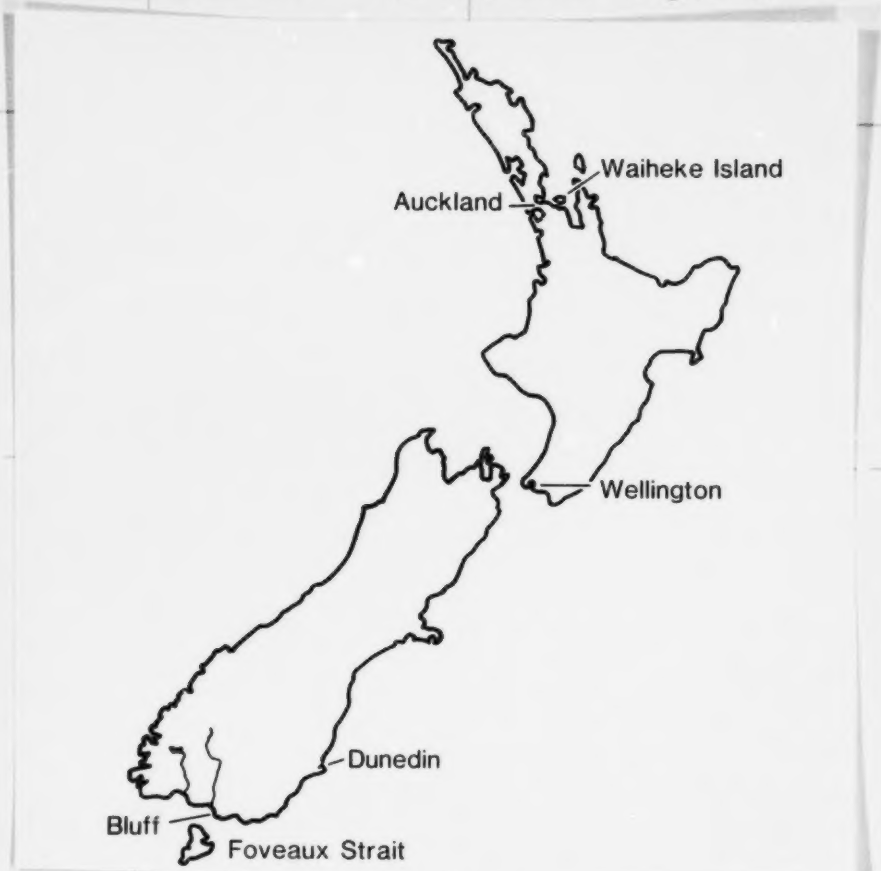

FIGURE 2: LOCATION AROUND THE NEW ZEALAND COAST OF SAMPLES OF MOLLUSCS ANALYSED FOR HEAVY METAL CONCENTRATIONS. (From Nielsen & Nathan, 1975).

In another study Nielsen and Nathan (1975) analysed cadmium, lead, mercury, zinc, copper and iron in 13 different species of shellfish from 199 different sites (Figure 2) and found that oysters accumulated approximately ten times more cadmium than the other species, except scallops. (Although scallops found in some areas of New Zealand contain high concentrations of cadmium, the metal is largely present in the visceral mass which is not eaten). Some of the results for cadmium are given in Figure 3. Ostrea lutaria is the dredge oyster which is fished at Bluff and consumed by the oystermen in our study. Crassostrea glomerata is the rock oyster common in the Auckland area. It is interesting to note that the dredge oysters in the Bluff area and the rock oysters at Waiheke Island, near Auckland, have very different distributions of the metals (Table 3).

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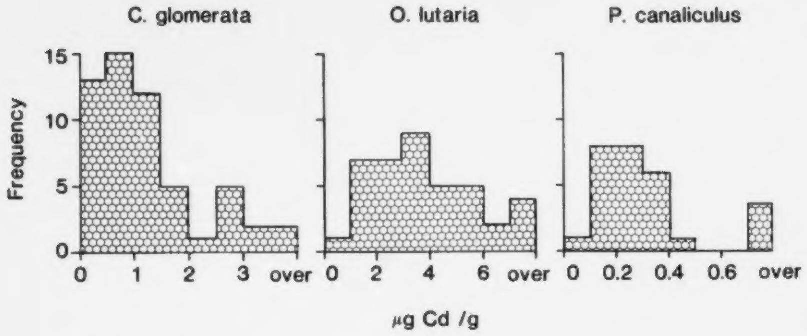

FIGURE 3: FREQUENCY DISTRIBUTIONS OF CADMIUM CONCENTRATIONS (µg/g wet wt) IN SAMPLES OF ROCK OYSTERS (CRASSOSTREA GLOMERATA), DREDGE OYSTERS (OSTREA LUTARIA) AND GREEN-LIPPED MUSSLES (PEREA CANALICULUS). (From Nielsen & Nathan, 1975).

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TABLE 3. COMPOSITION OF BLUFF OYSTERS COMPARED WITH WAIHEKE OYSTERS

	Metal concentration (µg/g wet weight)				
	Cd	Pb	Zn	Cu	Fe
Bluff, dredge oyster (n = 15)	5.2	0.45	60	13	40
Waiheke, rock oyster (n = 4)	1.0	0.5	441	25	18

People eating Waiheke oysters would get a much higher zinc and copper intake than those eating Bluff osyters at the same rate. This is intriguing, because differences in cadmium absorption or tissue accumulation between groups consuming different kinds of oysters could be caused by interactions from other metals in the oysters.

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A comparison of the normal daily intake of cadmium, zinc, copper, manganese and selenium, with the estimated intake from 1 Bluff oyster is presented in Table 4. Each Bluff oyster would more than double the daily cadmium intake, while for selenium each oyster would increase the normal intake by about 10%, and for the other trace elements by a much smaller proportion. From this data it can be calculated that 1 dozen oysters per week would be the maximum number which could be eaten in addition to the normal daily intake in order not to exceed the WHO Provisional Tolerable intake of 400-500 μg Cd/week (World Health Organization, 1972).

TABLE 4. COMPARISON OF THE TRACE ELEMENT CONTENT OF A NORMAL NEW ZEALAND DIET WITH THE CONTENT OF A BLUFF OYSTER

Element	Intake from "normal" New Zealand food	Intake per Bluff oyster (average weight 6 g)
Cd	15 μg (estimate of geometric mean from Guthrie & Robinson, 1977)	34.5 μg (average conc., 5.75 μg/g, wet wt., Nielsen, 1975)
Zn	10,000 μg (Guthrie & Robinson, 1977)	320 μg (average conc., 53 μg/g. wet wt., Guthrie, 1975)
Cu	1,500 μg (estimate of geometric mean from Guthrie & Robinson, 1977)	26 μg (average conc., 4.3 μg/g, wet wt., Guthrie, 1975)
Mn	2,700 μg (Guthrie & Robinson, 1977)	4.2 μg (average conc., 0.7 μg/g, (wet wt., Guthrie, 1975)
Se	30 μg (Griffiths, 1973)	2.8 μg (average conc., 0.46 μg/g, wet wt., Robinson, to be published)

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SECTION 4.

DEVELOPMENT OF METHODS FOR CADMIUM ANALYSIS

4.1. Introduction

Concentrations of cadmium in the body fluids of normal populations are very low. Whole blood levels are generally 3 µg/L or less while urine concentrations are less than 1 µg/L (Baker et al., 1979). Most published methods require extraction of cadmium from the biological matrix (Kjellstrom, 1979; O'Laughlin et al., 1976). Only a few direct determinations of low levels of cadmium in these matrices have been reported (Brodie, 1977; Pleban & Pearson, 1979). Sample contamination and matrix effects appear to cause significant problems in the attempt to determine accurately submicrogram levels of cadmium in biological matrices (Baker et al., 1979).

For the purposes of the present study a simple and sensitive method was therefore developed (Sharma et al., 1982) that minimised the risk of sample contamination and provided accurate results. It must be emphasised that to obtain accuracy it was necessary to follow all aspects of the analytical methods rigorously, particularly with regard to avoiding sample contamination, and to making volume measurements.

The method used for the analysis of faeces and oysters had already been developed and standardised and no further method development was considered necessary (refer to Section 5.3.).

4.2. Analysis of Cadmium in Whole Blood, Urine and Hair

4.2.1. Materials and Methods

Chemicals

Distilled deionised (Millipore, Redford, Massachusetts) water was used for preparation and dilution of all solutions.

Acids: The nitric and sulphuric acids used were high purity Ultrex grade obtained from J.T. Baker Chemical Company (Phillipsburgh, New Jersey).

Cadmium Standards: High purity cadmium (99.9998%) was obtained from Gluka AG., Buchs SF, Switzerland. A known weight (0.10148 g) of cadmium metal was dissolved in 5M nitric acid and the solution diluted to 100 ml with deionised water. The final concentration of cadmium in the standard stock solution was 1.0148 g/l. The standard was stored in a teflon bottle.

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APDC: 2 g ammonium pyrrolidenedithiocarbamate (Sigma Chemical Co., St Louis, Missouri) was dissolved in deionised water and the solution was made up to 100 ml, and stored in a polypropylene container.

MIBK: J.T. Baker analysed methyl-isobutyl-ketone was used as the extraction solvent.

Cleaning Procedure

All containers were soaked first in quadralene (Quadralene Products, England) overnight, rinsed in distilled water, and then soaked in 2M nitric acid for at least 4 hours, rinsed with deionised water and dried in a dust free oven. Immediately prior to use, the containers were rinsed with 0.5M nitric acid.

Sampling

Whole Blood: Approximately 15 ml whole blood was drawn into heparinized vacutainers. Two batches of sub-samples of 0.5 ml were taken in duplicate for analysis. A Gilson (Gilson France, Williers-Le-Bel, France) micropipette with disposable polypropylene tips was used for the sub-sampling. To avoid possible contamination from the pipette tips and to facilitate accurate, reproducible volume measurements the first intake of blood into the tip was discarded and the second intake was taken as the 0.5 ml sample.

Urine: The samples were collected in acid cleaned polyethylene containers. Sub-samples of 2.5 ml were taken for analysis as described above for the blood samples.

Hair: The samples were initially soaked in 1% non-ionic detergent Decon 75 (A. Gallenkamp & Co., London), rinsed with deionised water and then soaked in 0.1M solution EDTA for 30 minutes followed by rinsing with deionised water and drying. The hair was then finely cut with clean scissors, mixed thoroughly and then weighed directly (approx. 0.1 g) into digestion tubes.

Matrix Modification

Acid Digestion: The known volume or weight of the sub-samples was digested in Kimax (Kimble Division of Owens, Illinois) closed digestion tubes. All the samples were digested in 0.5 ml nitric acid at 70°C except hair, which was digested in a 1 ml mixture of 1:1 nitric and sulphuric acids. Although a low temperature necessitated longer digestion, the operation was conveniently carried out overnight. The digest was appropriately diluted and 10 µl of one batch of sub-sample digest solutions was injected into the graphite furnace for analysis.

Chelation and Extraction: To the second batch of sub-sample digests, 0.4 ml of 2% APDC solution and 2 ml of MIBK was added. The mixture was shaken for 5 minutes and allowed to stand for another 10 minutes to allow for the separation of the polar and non-polar phases. The tope non-polar phase was injected (10 µl) into the graphite furnace for analysis.

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Standard Addition Calibration

To four duplicate sub-samples of whole blood were added cadmium standards to give added concentrations of 0.25, 0.51, 0.76 and 1.01 ng Cd/ml in the final analytical volume. A fifth duplicate sample was used as the control. All the samples were digested, diluted to 5 ml and analysed for cadmium. Similar studies were carried out for hair and urine samples.

Recovery Studies

Five urine samples were divided into two batches of five duplicate samples. One batch was digested and analysed for cadmium. The second batch was spiked with known amounts of cadmium, digested and then analysed for cadmium. The concentrations of cadmium were calculated with reference to the corresponding standard addition calibration curve and the recoveries determined. Similar studies were carried out for hair and whole blood samples.

Comparison of digest injection and APDC/MIBK extraction methods

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Hair samples: Samples were divided as for recovery studies described above and digested. One batch of samples was diluted and analysed for cadmium. The other batch was treated with APDC and MIBK, and the solvent extract analysed for cadmium. The relative standard deviation (RSD) values given for each sample were calculated from twelve injections (i.e. duplicate samples two sets of six injections).

Instruments

An IL (Instrumentation Laboratories, Lexington, Massachusetts) 351 Atomic Absorption Spectrophotometer with an IL 555 CTF Flameless Atomiser was used for the analysis of Cd in blood and urine. The high speed IL 114 recorder was used for determining the optimum time-temperature programme for the flameless atomiser. A deuterium continuum source was used for simultaneous background correction. A Varian Techtron AA 775 spectrophotometer with flameless atomiser (CRA-90) was used for analysing Cd in the hair samples.

4.2.2. **Flameless Atomiser Programme**

In flameless atomic absorption analysis, the sample application in terms of spreading and absorption onto the graphite, and the temperature-time programming in terms of sample splattering (drying stage) and loss of analyte (ashing stage) before atomisation, influence significantly the overall precision and accuracy (Garnys & Smythe, 1975). These factors were considered very carefully in experimentally determining the spectrophotometer and flameless atomiser parameters (Table 5) for the analysis of cadmium. Two-stage drying and ashing with a low heating rate was used to eliminate sample splattering and to achieve optimum matrix pyrolysis without the loss of cadmium prior to atomisation. A ramp atomisation with a 10 sec hold was used to obtain optimum peak height sensitivity with minumum residual build-up.

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TABLE 5. SPECTROPHOTOMETER AND FLAMELESS ATOMISER PARAMETERS USED FOR CADMIUM ANALYSIS IN BLOOD, URINE AND HAIR

A: SPECTROPHOTOMETER PARAMETERS

Instrument	Wavelength nm	Current mA	Bandpass	Purge Gas
IL 351	228.8	2	1	Argon
Varian 775	228.8	2	1	Argon

B: FLAMELESS ATOMISER PARAMETERS

Instrument and Stage		Temperature °C	Time sec
IL 555	Dry I	75	20
	II	130	20
	Ash I	300	30
	II	350	10
	Atomise I	2000	0
	II	2000	10
Varian CRA	Dry	95	40
	Ash	350	15
	Atomise	1500	10

As an example, a complete dry, ash and atomise cycle with and without background correction is shown in Figure 4 for the analysis of cadmium in a whole blood digest. It is clear from the trace that most of the sample matrix was pyrrolised before atomisation, and the residual non-specific absorption during the atomisation stage was readily corrected for by the simultaneous background correction system. The background corrected trace (Figure 4 (b)) indicates that there was no detectable cadmium loss during the ashing stage. Addition of ammonium dihydrogen orthophosphate to the sample digest allowed the use of a higher ashing temperature (up to 450°C); however, the addition was considered undesirable due to the cadmium present in the Analar grade compound available.

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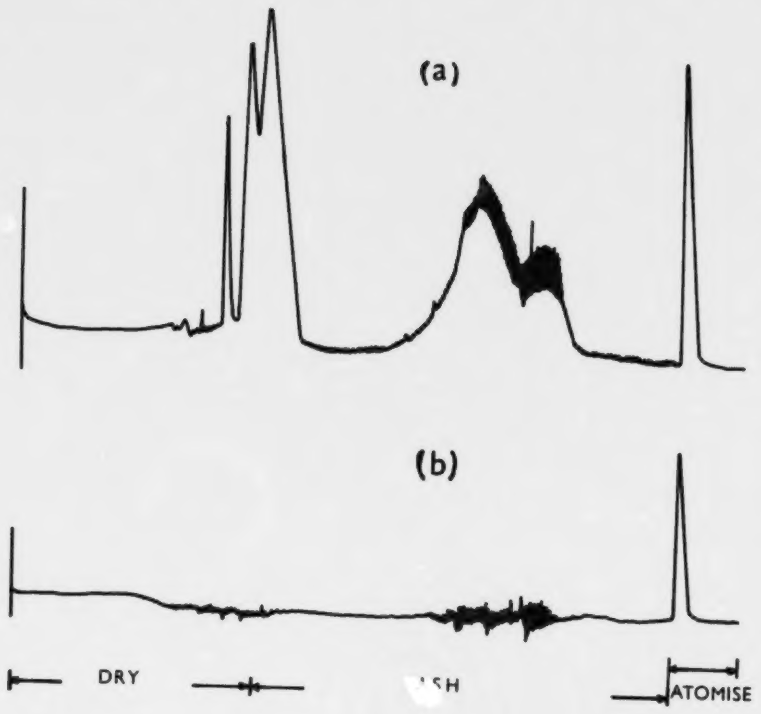

FIGURE 4: RECORDER SHOWING TRACES FOR CADMIUM ANALYSIS IN A WHOLE BLOOD DIGEST
(a) A BACKGROUND UNCORRECTED TRACE AND
(b) A CORRECTED TRACE
OVER THE WHOLE TIME-TEMPERATURE PROGRAMME CYCLE. THE ATOMIC PEAK REPRESENTS A CADMIUM CONCENTRATION OF 1.02 ng/ml.

4.2.3. Cadmium Calibration Curve & Recovery of Added Cadmium

The standard addition calibration technique was used for the determination of cadmium in the samples. A typical calibration graph for whole blood samples is shown in Figure 5. The direct digest injection method was used for the cadmium analysis. Similar graphs were obtained for the determination of cadmium in the hair and urine samples. Even though most of the sample matrix was oxidised and eliminated before atomisation it was

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still considered necessary to use standard addition method to take into account any possible effects of residual substances on the atomisation of cadmium.

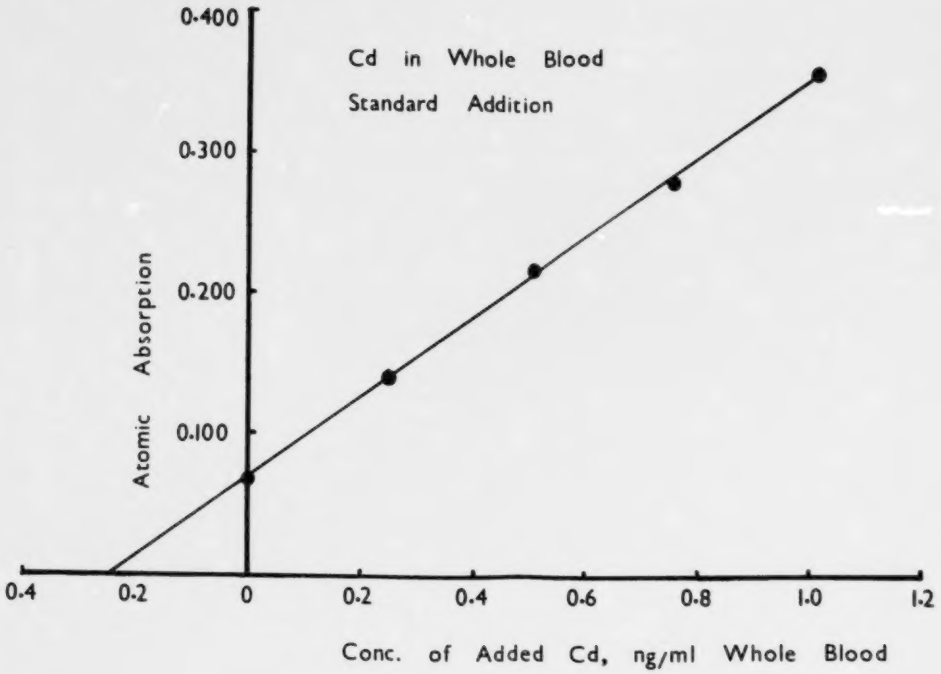

FIGURE 5: A TYPICAL STANDARD ADDITION CALIBRATION GRAPH USED FOR DETERMINING THE CONCENTRATION OF CADMIUM IN WHOLE BLOOD.

The results of recovery studies carried out with urine samples spiked with known amounts of cadmium are shown in Table 6. The digest injection method used gave an average recovery of 99%; the overall recovery for all samples (blood, urine and hair) was 99%.

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TABLE 6. TYPICAL RECOVERIES OF CADMIUM ADDED TO URINE SAMPLES

Sample	Cd Conc. ng/ml	Cd Added ng/ml	Cd Conc. in spiked sample ng/ml	% Recovery
U(1)	7.26	2.03	9.24	97
U(2)	5.90	2.03	7.91	99
U(3)	5.67	2.03	7.72	101
U(4)	5.90	1.01	6.89	98
U(5)	5.79	1.01	6.82	101
			Mean:	99

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To check further the accuracy of the results obtained by the digest injection method, duplicate samples were analysed following APDC/MIBK chelation and extraction. The results from the analysis of cadmium in the whole blood of 25 people is shown in Figure 6. There were no significant differences in the blood cadmium levels obtained by the two methods; the cadmium concentration values from the extraction method were on average slightly lower.

4.2.4. Precision and Reproducibility

The analytical precision (manual injection) achieved using the digest injection method is shown in Table 7. For comparison the samples (in this example, hair) were analysed by the extraction method also and the corresponding relative standard deviation (RSD) values are shown in the table. The overall average RSD for the digest injection method was 2.4% and for the corresponding APDC/MIBK extraction method, 9.4%. The relatively high RSD for the extraction method was due mainly to the poor reproducibility of sample injections. The organic solvent (MIBK) had a tendency to creep and to dispense unevenly in the graphite tube. Flame analysis could have been used for the determination of cadmium in the MIBK extract, however the small sample size (about 0.1 g hair) resulted in cadmium concentrations in the final analytical volume (2 ml) that were beyond the detection limit of the flame method.

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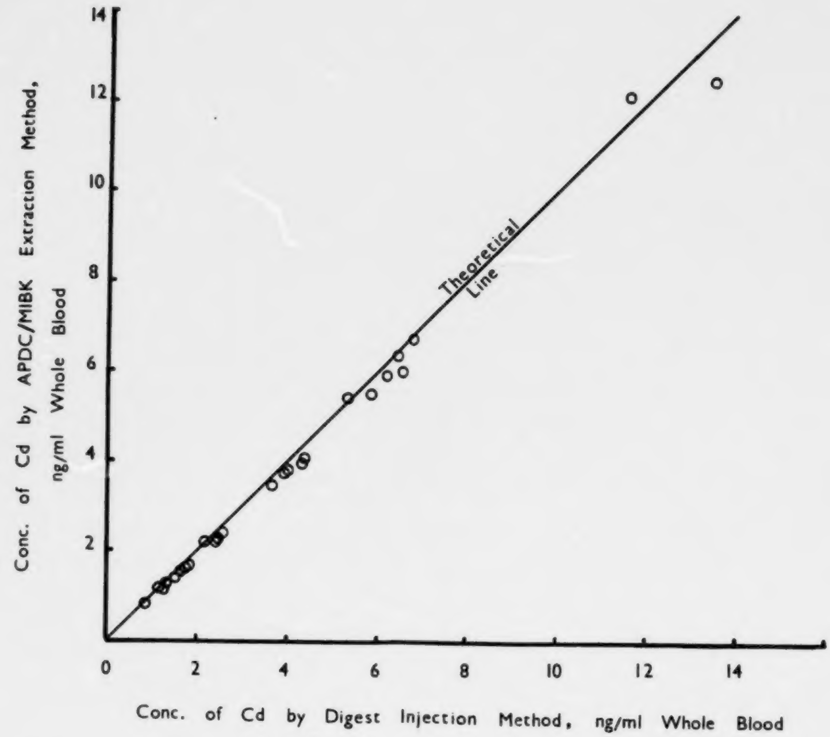

FIGURE 6: DETERMINATION OF CADMIUM IN WHOLE BLOOD BY THE DIGEST INJECTION METHOD AND APDC/MIBK CHELATION AND EXTRACTION METHOD. DUPLICATE SAMPLES WERE ANALYSED BY THE EXTRACTION METHOD AS A CROSS-CHECK ON THE DIGEST INJECTION METHOD.

TABLE 7. ANALYSIS OF CADMIUM IN HAIR BY THE DIGEST INJECTION METHOD AND BY APDC/MIBK EXTRACTION

Sample	Digest Injection Method ng Cd/g	RSD %	Chelation and Extraction Method ng Cd/g	RSD %
H(1)	71.28	1	77.91	8
H(2)	838.80	3	801.89	12
H(3)	213.87	1	207.03	9
H(4)	278.84	4	271.59	8
H(5)	908.67	3	882.32	10

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The reproducibility of the analytical methods described was also checked. As an example the results of three different analyses (Digests A, B and C) of the same hair sample, performed independently at different times, is shown in Table 8. The weight of hair taken for each analysis was 0.1724, 0.1036 and 0.1060 g, respectively, for digests A, B and C; each diluted digest had a final volume of 10 ml. The mean concentration of cadmium in the hair sample was 0.878 ± 0.008 µg/g. The results indicated a good reproducibility for the analytical methods used.

TABLE 8. REPRODUCIBILITY OF RESULTS BY THE STANDARD ADDITIONS METHOD

	Replicates of Sample Digest				
	1	2	3	4	5
Volume (ml)	0.5	0.5	0.5	0.5	0.5
Cd Added (ng/ml)	0.0	0.507	1.014	1.522	2.029
Diluted Volume (ml)	10	10	10	10	10
Absorbances for Digest A	0.142	0.258	0.361	0.443	0.513
Absorbances for Digest B	0.091	0.198	0.313	0.404	0.408
Absorbances for Digest C	0.099	0.221	0.311	0.407	0.488

Digest	Regression Coefficient	Standard Addition Slope Line	Hair Cd ng/g
A	0.997	0.198	0.879
B	0.999	0.207	0.867
C	0.997	0.191	0.887

4.3. Interlaboratory Comparisons

Duplicate specimens of whole blood, faeces and hair were sent to Dr Kazuo Nomiyama (Department of Environmental Health, Jichi Medical School, Tochigi-Ken Japan) for cross-check analysis. The results for the blood and faeces specimens compared well (Table 9); however the concentrations for the hair specimens were different. It was noted that at JMS the hair specimens were dried for a further period of 2 days in a 80°C oven. No additional drying was carried out at the Toxicology Unit. This difference in sample treatment together with the dry ashing as opposed to wet ashing could have accounted for the lower concentration of cadmium in the hair specimens determined at JMS.

TABLE 9. COMPARISON OF ANALYTICAL METHODS AND RESULTS FROM JICHI MEDICAL SCHOOL (JMS) AND THE TOXICOLOGY RESEARCH UNIT (TRU)

	JMS	TRU
Sample prepration	Dry ashing	Wet ashing
Instrumentation	Zeeman-effect Flameless AAS	Flameless AAS with D_2 simultaneous background correction
Whole blood result	3.13 ng/g	2.85 ± 0.21 ng/g
Faeces result	3.4 ng/g	4.05 ± 0.16 ng/g
Hair result	0.53 µg/g	0.84 ± 0.03 µg/g

Attempts to compare our atomic absorption for blood method with neutron activation analysis failed because the neutron activation analysis was not sensitive enough and gave results at the detection limit (1 ng/ml).

4.4 Summary

Sample contamination and/or loss of the analyte are the main contributors to errors incurred during sample preparation in the determination of low levels of cadmium. The results presented demonstrate that the sampling and digestion procedure described in the methods, provided two distinct advantages:

1. The closed system for the digestion tube avoided contamination from dust particles; appropriate dilutions were carried out in the same tube (less sample handling).

2. Being a closed pressurised system it provided a more efficient digestion, requiring a small amount of the high purity (expensive) acid.

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The methods described for the analysis of low levels of cadmium in the various biological matrices were believed to have eliminated sample contamination and/or cadmium loss and to have given precise and accurate results. Furthermore the digest injection method was found to be simple and highly reproducible.

This section described the work to develop the methods for cadmium analysis. During the routine analysis phase of the study further checks of recovery, precision and accuracy were carried out (Section 5.3).

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SECTION 5

MATERIALS AND METHODS FOR THE POPULATION STUDY

5.1. Epidemiological design

The township of Bluff, where the oyster fishing industry is based, has a total population of 1700 people, many of whom have lived in the township for most of their lives. Bluff is the southernmost township on the South Island of New Zealand, and is situated around a natural harbour which handles a high density of domestic and international shipping (Figure 7). The other main industry in Bluff is a meat industry slaughtering works. On the opposite side of the harbour from Bluff township is a 2 pot-line aluminium smelter; 10 miles from Bluff is a superphosphate manufacturing plant for which the phosphate rock is shipped into Bluff and then transported by road to the works.

Foveaux Strait lies between Bluff and an off-shore island, Stewart Island. The oyster beds are the only naturally occuring deep sea oyster beds still commercially fished in the world. The main surface flow through Foveaux Strait is west-east, and oysters at the western end of the oyster beds have a higher cadmium content than do the eastern oysters, or the rock oysters found on Stewart Island. The permitted seasonal catch is 115,000 sacks of oysters; 1 sack contains an average of 70 dozen oysters,. Therefore the seasonal catch is about 100 million oysters, i.e. 32 oysters for each New Zealander. Only 1% of each season's catch is exported.

About 70 male and female workers are employed in the oyster canning industry and 50 male fishermen are involved full time in the fishing during the season from March to August. In addition, there are 50 retired workers from this industry still living in the same area. Meetings were held with the oysterfishermen and canning workers in the fishing port of Bluff before the season started in 1981, and everyone was encouraged to take part in the study. As the study involved the collection of faeces, urine, blood and hair samples as well as keeping dietary records, etc., it was not expected that everyone would be willing to participate.

The aim was to find a same-sized reference group of meat industry slaughtermen with a low oyster intake, but it turned out that many of these workers in the area also had a high oyster intake, and furthermore, it was even more difficult to convince them to take part as they had no personal involvement with the oyster industry.

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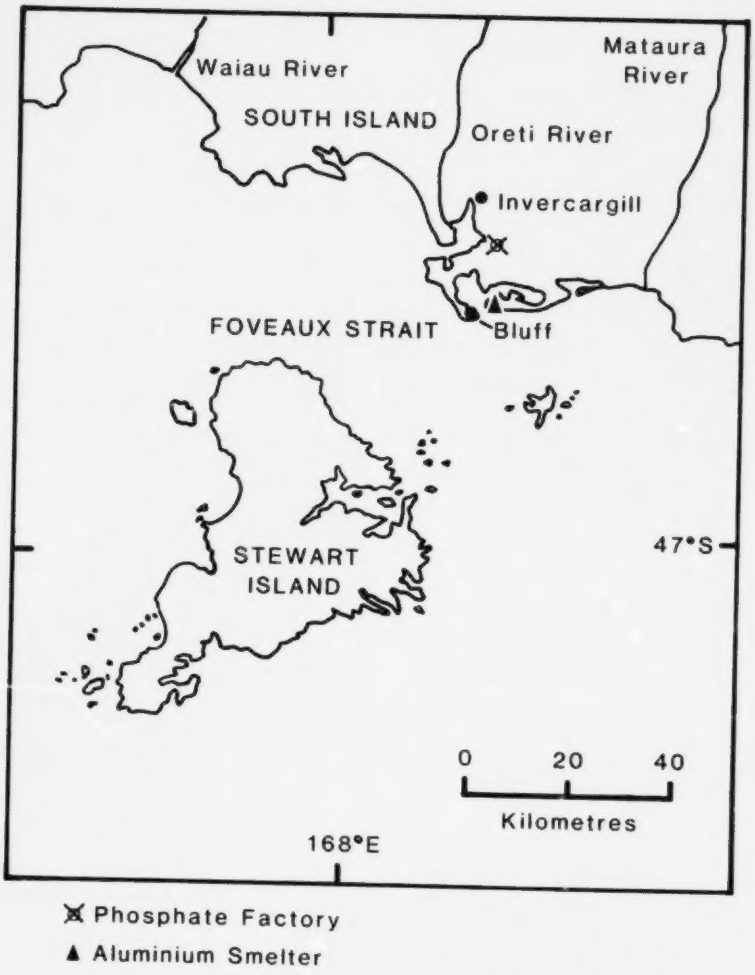
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SOUTH ISLAND
Oreti River
Mataura River
Invercargill
Bluff
FOVEAUX STRAIT
STEWART ISLAND
47°S
168°E
0
20
40
Kilometres
☒ Phosphate Factory
▲ Aluminium Smelter

FIGURE 7: MAP OF BLUFF AND SURROUNDING AREAS.

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A number of people were found in Bluff and in small communities adjacent to Bluff with a low oyster intake but with similar living conditions, etc., to the oyster fishermen; these people were included in the group as a reference. All in all, 76 people were selected and they were classified according to average oyster consumption during the season into 4 groups. All these people lived in the Bluff or Invercargill areas. An additional reference group for the study of blood cadmium and urine cadmium was found in the city of Dunedin.

Samples were collected at two times, and for a subgroup of 18 people at three times. The first samples of faeces, urine, blood and hair were collected in the week before the season started on 1 March 1981. However the equipment on the oyster fishing boats is checked in the presence of a Ministry of Agriculture and Fisheries Inspector in the few days preceeding the opening of the season. Since the oysters reputedly taste better at the beginning of the season some fishermen had already eaten a few oysters in that week preceeding the official opening of the season, and their faecal output of cadmium reflected that intake. In the middle of the season in July 1981 additional samples of blood and a faecal specimen were collected from eighteen people who were keen to cooperate; they also kept a daily diary of oyster intake from then until the next sampling. Finally, at the end of the season in August 1981 a full set of faeces, urine, blood and hair samples were collected again, as well as an interview to obtain a dietary history and a medical history. The analyses carried out are described in the next section (5.2.).

With this design of the study, we could evaluate the relationship between oyster intake and cadmium intake on a quantitative basis and we could study its time relationship, because at the beginning of the season the intake was likely to be less than in the middle or the end of the season.

The group studied covered the age range 20-75 years and it included 57 men and 19 women. Since we were describing mainly the short term relationship between cadmium intake and cadmium in tissues it was considered that any age effect on the cadmium concentration in tissues was not likely to cause bias in the results.

In order to evaluate the possible effects of cadmium, urinary beta-2-microglobulin as well as total proteinuria and glycosuria were measured, blood pressure was measured and the medical history was recorded in an interview.

5.2. Sample and data collection

Protocols for the handling of samples were drawn up for each series of sample collections (Appendix A). The survey team resided in Bluff for the week required to obtain samples at each visit, and used the Ministry of Agriculture and Fisheries research laboratory for the immediate handling and necessary processing of samples. All of the methods described here have been standardised for trace element analysis within the Nutrition Department and validated by years of use in metabolic balance studies and population surveys.

5.2.1. Sample Collection during the Survey

(a) Faeces. Subjects were asked to collect all faeces passed on the 3 days of their study period; samples initially were collected and stored individually. Waxed milkshake containers (500 ml) lined with a trace element free plastic bag were used for collection; the containers were then sealed in outer plastic bags, and handed in for frozen storage on the day of collection. A few subjects preferred to collect faecal specimens directly into large plastic rubbish bags which were spread over the toilet seat. Three subjects failed to produce any faeces during the pre-season collection, and five subjects did not produce any faeces during the end-season collections.

(b) Urine. Subjects were given written instructions which were explained to them on how to collect an overnight specimen (Appendix B). Urine samples were collected into weighed acid-washed plastic bottles; women collected urine first into a plastic basin and then transferred the sample using a plastic funnel. The urines were processed on the same day of collection by measurement of pH, specific gravity, and labstix analysis for glucose or protein. An aliquot was transferred into polyethylene test tubes containing sodium bicarbonate for later analysis for beta-2-microglobulin concentration, and the urine samples were then sealed into their collection bottles, packed into cartons so that they remained upright, and transferred to refrigerated storage within 10 hours of completion of collection.

(c) Blood samples. Blood was drawn by venipuncture into heparinized or plain vacutainer tubes (Becton-Dickinson). Within 2 hours PCV was measured and the clotted blood was spun to obtain serum. Aliquots were taken for subsequent analysis and samples were stored at 5°C.

(d) Hair samples. Scalp hair was clipped from the occipital region, as close as possible to the scalp. The cut end of the hair sample was identified before sealing in a plastic bag, and subjects were asked what hair shampoo they had last used.

5.2.2. Data Collection and Questionnaires

(a) Pre-season. Each subject was interviewed to obtain information on their age, occupation, smoking habits, and history of oyster consumption including when they had last eaten oysters (and how many). (See Appendix C for Interview Sheet).

(b) Mid-season. Subjects were again asked when they had last eaten osyters, and they were given dated notebooks to record each days oyster consumption

(c) End-season. (i) Dietary History: Each subject was administered (by the same interviewer, JM) a detailed dietary history questionnaire, including questions on their history of fish and shellfish consumption (See Appendix D).

(ii) Medical History: Each subject was administered a medical history questionnaire (by the same interviewer, TK) to determine the incidence of chronic diseases, and history of specific diseases (renal, skeletal). (See Appendix E). At the same time their height (with shoes) and weight (with clothes and shoes) were measured using portable survey equipment, and one sitting blood pressure measurement was taken (TK).

5.2.3. Sample Transportation and Initial Processing

Urine and blood samples, and faecal samples were transported refrigerated and frozen, respectively, the following week to the laboratories in Dunedin.

(a) Faeces. Individual samples were removed from the plastic bags and weighed into porcelain or pyrex drying dishes while still frozen. They were dried to constant weight under ultra violet lamps, ground, and the total 3-day sample for each person was pooled and ground to a fine powder. Samples were stored in air-tight polyethylene jars until analysis.

(b) Urine. The samples were allowed to return to room temperature, and then aliquots were taken for autoanalyzer analysis for creatinine (pre- and end-season samples) and for urea analysis (end-season samples). Further aliquots of urine were taken for zinc analysis, for cadmium analysis, and for storage, and the remainder of the sample was discarded. Aliquots were stored until analysis either refrigerated or frozen.

(c) Blood samples. Heparinized whole blood samples were aliquoted for immediate analysis of haemoglobin content, and for later analysis for whole blood selenium and cadmium concentrations. Bloods were stored refrigerated or frozen as required until analysis.

(d) Hair samples. No initial processing was required until washing before analysis.

5.3. Analytical Methods

5.3.1. Faeces Analysis: Cadmium, Zinc, Copper, Manganese

Faeces were analysed by the method of dry ashing which has been employed for trace element analyses in the Nutrition Department for 12 years (Robinson, McKenzie, Thomson & van Rij, 1973; McKenzie, 1973). Duplicate samples of about 5 g were weighed accurately into pyrex beakers and ashed in muffle furnaces at increasing temperatures up to 450°C. Silica lids were in place on the beakers at all times in the furnaces. The ash was cooled, dissolved in 2 ml conc. HCl (Baker Analar), heated to boiling point with a watch glass covering the beaker, and then transferred quantitatively with hot 0.1 M HCl to 25 ml volumetric flasks through Whatman #540 Ashless filter paper. The solutions were cooled, and diluted to volume with Milli-Q water. Cadmium was read directly on the solutions by flame atomic absorption at 224.8 nm; copper and manganese were read on 1:10 dilutions at 213.9 and 279.5 nm, respectively, and zinc was read on 1:100 dilutions at 213.8 nm. All readings

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were compared against the absorbances of appropriately diluted standard solutions prepared from Fisher Atomic Absorption 1,000 ppm Standard Solutions.

Replicate analyses of a stock faeces sample and recovery of element added before the ashing of the sample was good by this technique, as shown by the example given for precision and recovery of cadmium, zinc, copper and manganese in Table 10. Reproducibility of the analysis of a stock liver sample by this method over a four-year period is presented in Table 11.

TABLE 10. REPEATABILITY AND RECOVERY OF CADMIUM, ZINC COPPER AND MANGANESE IN A STOCK FAECES SAMPLE

Element	No. Replicates	Precision of Analysis, μg/g DM $\bar{x} \pm$ SD	Recovery of Added Element, % $\bar{x} \pm$ SD
Cadmium	6	0.40 ± 0.03	96 ± 6
Zinc	6	557 ± 8	103 ± 2
Copper	6	231 ± 8	98 ± 4
Manganese	6	263 ± 4	101 ± 3

TABLE 11. RESULTS FOR A STOCK LIVER SAMPLE ANALYSED BY DRY ASHING OVER A 4-YEAR PERIOD (mean ± SD)

Element	No. Replicates	Analytical Result μg/g DM	Recovery of Added Element %
Cadmium	32	0.15 ± 0.02	
	12		94 ± 7
Zinc	36	112 ± 9	
	19		100 ± 3
Copper	40	25.4 ± 1.7	
	19		102 ± 6
Manganese	31	9.4 ± 0.7	
	19		102 ± 5

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Analysis of National Bureau of Standards Reference Material #1577 (Bovine Liver) by this method gave results closely comparable to the certified values as shown in Table 12.

TABLE 12. RESULTS OF ANALYSIS OF NBS STANDARD REFERENCE MATERIAL #1577 (BOVINE LIVER) COMPARED WITH THE CERTIFIED VALUES

Element	No. Replicates	Analytical Result µg/g DM	Certified Value µg/g DM
Cadmium	6	0.28 ± 0.02	0.27 ± 0.04
Zinc	6	130 ± 3	130 ± 10
Copper	6	180 ± 1	193 ± 10
Manganese	6	10.2 ± 0.3	10.3 ± 1.0

5.3.2. Whole Blood and Serum Analysis: Packed Cell Volume; Haemoglobin; Whole Blood Cadmium; Whole Blood Selenium; Serum Zinc and Copper; Serum Beta-2-microglobulin

(a) Packed cell volume. Measurements were made on fresh heparinized samples using a microcentrifuge and standard techniques.

(b) Haemoglobin. Measurements were made on heparinized samples using Drabkins reagent and taking absorbance measurements after 20 minutes on a Unicam spectrophotometer at 542 nm.

(c) Whole blood cadmium. Refer to Chapter 4.

(d) Whole blood selenium. The fluorimetric assay using diaminonaphthalene has been standardised in the Nutrition Department (Thomson, 1973), and an automated procedure was developed subsequently. Triplicate estimations using 1 ml blood were carried out for each sample. Reproducibility of estimations and recovery of added selenium using this assay are as shown in Table 13.

(e) Serum zinc and copper. Estimations were performed in duplicate on 1:10 dilutions with 6% butanol and compared against appropriately diluted standards (McKenzie, 1979). Repeatability of the assay and recovery of added zinc and copper are presented in Table 14. The results for a reference simulated serum (Cation Cal) analysed by the same method were for zinc 290 µg/100 ml compared with a certified value of 315 µg/100 ml, and for copper 215 µg/100 ml compared with the certified value of 208 µg/100 ml.

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(f) Serum beta-2-microglobulin. Radioimmunoassay kits (Pharmacia) were used for beta-2-microglobulin assays.

TABLE 13. PRECISION OF THE FLUORIMETRIC ASSAY FOR SELENIUM AND RECOVERY OF ADDED SELENIUM

Sample No.	No. Replicates	Analytical Result μg/ml	Recovery of Added Selenium %
1	9	0.045 ± 0.001	
2	10	0.050 ± 0.002	
3	12		99.9 ± 1.4

TABLE 14. PRECISION OF THE ESTIMATION OF ZINC AND COPPER IN SERUM AND RECOVERY OF ADDED ZINC AND COPPER

Element	No. Replicates	Analytical Result μg/100 ml	Recovery of Added Element %
Zinc	8	123 ± 3	102 ± 3
Copper	8	107 ± 2	98 ± 1

5.3.3. Urine Analysis: pH; specific gravity; cadmium; zinc; creatinine; urea; glucose and protein; beta-2-microglobulin

(a) pH. The pH of fresh pre-season urine specimens was measured using Combur 8 Test strips (Boehringer); for end-season urines pH was measured on fresh samples using a Solstat EPM 90 portable pH meter.

(b) Specific gravity. Specific gravity was measured on fresh urine samples using a portable refractometer.

(c) Glucose and Protein. Combur 8 Test strips (Boehringer) dipped into fresh urine were compared against the appropriate colour scale.

(d) Creatinine and Urea. Creatinine only was measured on pre-season urine samples; both creatinine and urea were estimated by auto-analyser (Chem Lab) in the Dunedin Public Hospital Chemical Pathology Laboratory in the end-season samples.

(e) Cadmium. Refer to Section 4.

(f) Zinc. Well-mixed aliquots of urines were sprayed directly into the atomic absorption spectrophotometer and compared against aqueous standards. Repeatability of zinc analysis by this method was ±1% on 100 urine samples; recovery of added zinc for 20 recovery analyses was 102 ± 2%.

(g) Beta-2-microglobulin. Radioimmunoassay kits as for serum were used for end-season samples.

5.3.4. Hair Analyses : Cadmium; Zinc; Copper

The length of hair analysed was on average the proximal 2 ± 1 cm and the mean weight of hair analyzed was 22.5 ± 9.5 mg. The washing procedure standardised for flame atomic absorption analysis for zinc and copper (McKenzie, 1978) was used. Refer to section 4 for details of the analytical method. Duplicate analysis of eleven hair samples on two different days gave the results presented in Table 15.

TABLE 15. DUPLICATE ANALYSIS OF HAIR SAMPLES FOR CADMIUM, ZINC AND COPPER

Element	First Analysis µg/g DM	Duplicate Analysis
Cadmium	0.29 ± 0.20	0.30 ± 0.24
Zinc	174 ± 17	179 ± 28
Copper	22.2 ± 8.1	19.6 ± 7.1

5.3.5. Dietary Analysis: Energy; Protein; Calcium; Zinc; Iron

Using the information obtained on the dietary history questionnaires, each subject was categorised as having a low, medium or high intake of energy, protein, calcium, zinc and iron according to the criteria listed below. Cadmium was not included in this type of classification because of the over-riding influence of oyster consumption on dietary cadmium intake; each category of oyster intake therefore also represented categories of cadmium intake (Category I: "low"; Category IV: "very high"). Reference information

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such as percentage principal sources of nutrients in the diet of New Zealand residents was obtained from the 1977 National Diet Survey (Birkbeck, 1979).

(a) Energy. Proportionate energy sources in the New Zealand diet: fat 41-43%; carbohydrate 38-41%; protein 13-14%; ethanol 2-6%. Classification of the dietary histories was therefore made according to the following:-

Very High Intake: a high fat, carbohydrate and protein intake e.g. beef several times per week; cream or ice cream several times per week.

High-Medium Intake: a medium fat and carbohydrate intake e.g. meat once or twice per week.

Low Intake: a low fat, carbohydrate and protein intake.

(b) Protein. Percentage principal sources in the New Zealand diet: meat 45-50%; dairy products 12-17%; bread 13-15%. Classification of the dietary histories was therefore made according to the following:-

Very High Intake: meat and bread every day, as well as cheese, milk, eggs several times per week.

High-Medium Intake: meat several times per week, bread several times per week, occasionally cheese, milk, eggs.

Low Intake: meat occasionally; bread several times per week; dairy products and eggs occasionally.

(c) Calcium. Percentage principal sources in the New Zealand diet: dairy products 41-58%; bread 12-17%; vegetables 8-12%. Classification of the dietary histories was therefore made according to the following:-

Very High Intake: dairy products daily, bread daily, vegetables daily.

High-Medium Intake; milk daily; cheese several times per week; bread several times per week, vegetables daily.

Low Intake: no cheese or milk eaten; bread once or twice per week; vegetables less than daily.

(d) Zinc. Percentage principal sources in the New Zealand diet: meat 43-51%; dairy products 13-17%; bread 11-14%; vegetables 7-9%. Classification of the dietary histories was therefore made according to the following:-

Very High Intake: meat daily; dairy products daily; bread daily; oysters frequently.

High-Medium Intake: meat less than daily; dairy products daily; bread daily; oysters occasionally.

Low Intake: meat occasionally; dairy products once or twice per week; bread several times per week; no oysters.

(e) Iron. Percentage principal sources in the new Zealand diet: meat 40-47%; bread and cereals 15-19%; vegetables 9-13%. Classification of the dietary histories was therefore made according to the following:-

Very High Intake: meat daily; bread and cereals daily; vegetables daily; oysters frequently.

High-Medium Intake: meat several times per week, bread several times per week occasionally cheese, milk, eggs.

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Low Intake: meat occasionally; bread and cereals once or twice per week; vegetables once or twice per week.

5.3.6. Oyster Analysis: Cadmium; Zinc; Copper; Manganese; Selenium

Oysters were obtained fresh from the oyster opening factories, and then frozen until analysis. Water content was estimated by drying to constant weight in a vacuum oven; the dried sample was then ground and stored in an air-tight polyethylene container until dry ashing as for the faeces. A sample of six dozen oysters was taken for analysis for the 1981 season. Results reported in Section 3.

5.3.7. Statistical methods.

The distributions of the analytical results within groups were assumed to follow normal distributions. For some variables, e.g. faecal cadmium at the end of the oyster season, the distributions were very skewed and would fit better to a log-normal distribution. However, we assumed normal distributions for all estimates in this report to make them comparable with other published data.

Differences between means of groups were tested with Student's t-test. Correlation analysis was used to compare two sets of data from the same individuals. Results of the student's T-test are only indicated in the tables when the differences were "statistically significant" ($p>0.05$).

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SECTION 6

RESULTS

In total, 78 subjects took part in this survey; 64 subjects participated in the pre-season sampling; 18 subjects participated in the mid-season subsampling, and 62 subjects participated in the end-season sampling. Some of the subjects who participated in the pre-season sampling did not participate in the end-season sampling for various reasons (e.g. moved residence; refused to participate) so that the total number of subjects who participated in both the pre-season and the end-season sample was 48. A few subjects were of mixed Maori-Caucasian descent, but there were no full-blood races other than Caucasian in this study. The mixed racial ancestry of some subjects was not considered to be a factor influencing any of the findings or conclusions.

6.1. Dietary and Anthropometric Data

(a) Oyster intake and classification into oyster intake categories.

Subjects were questioned personally more than once about their average intake during the oyster season (March-August), and for most subjects their intake generally decreased as the season proceeded. From this information on oyster intake, as well as from the postal questionnaire a consensus figure for average oyster intake in dozen/week was derived (Appendix F). The highest average intake was 16 dozen/week; only two subjects said that they never ate oysters. From the distribution of oyster intakes (Figure 8) the subjects were then classified into four categories of oyster intake: Category I: 0-<$\frac{1}{2}$ dozen/week, n=18; Category II: $\frac{1}{2}$-<2 dozen/week, n=24; Category III: 2-<6 dozen/week; n=23; Category IV: $\geqslant 6$ dozen/week, n=12 (Table 16).

At the end of the season subjects were also asked when they had last eaten oysters, and how many, so that their intake corresponding to their end-season faecal collection could be compared with the average intake for the season (Table 16). For subjects in Category IV in particular their end-season oyster consumption was lower than the stated average consumption for the season.

(b) Nutrient intakes according to the Dietary Histories.

Data from the 1977 National Diet Survey was obtained by an activity-associated one day diet recall, and therefore could not be compared directly with the data obtained in the present study. Only two subjects (who participated only in the pre-season sampling)

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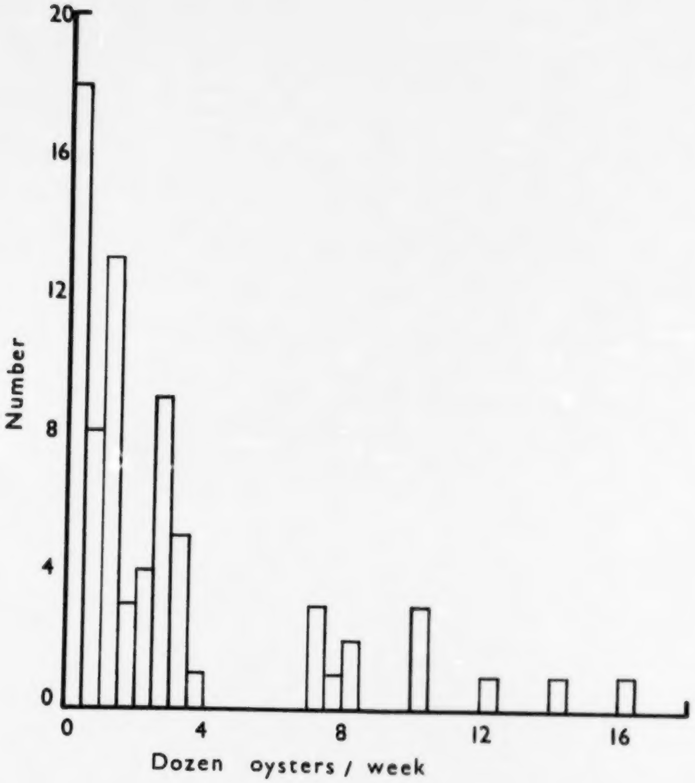

FIGURE 8: DISTRIBUTION OF AVERAGE SEASONAL OYSTER INTAKES FOR ALL SUBJECTS

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described themselves as vegetarians; they were (22.3.DC and 23.1.KC). All other subjects answered that they were not on any special type of diet, except for 13.3.RB who was on a low fat/low cholesterol diet, and 45.1.RK who was on a diabetic diet. The distributions of intake categories for end-season subjects (n = 58) are summarised in Table 17.

TABLE 16. COMPARISON OF AVERAGE OYSTER CONSUMPTION FOR THE SEASON WITH END-SEASON INTAKE

OYSTER INTAKE CATEGORY	OYSTER CONSUMPTION		
	Season Average dozen/week	Season Average oysters/day	End-Season oysters/day
I	0 - <½	0.7	0.8
II	½ - <2	1.9	2.5
III	2 - <6	5.2	4.2
IV	≥6	18.2	9.7

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TABLE 17. DISTRIBUTION OF VERY HIGH, HIGH-MEDIUM AND LOW INTAKES OF ENERGY, PROTEIN, CALCIUM, ZINC AND IRON OBTAINED FROM THE DIETARY HISTORY QUESTIONNAIRES

NUTRIENT	PERCENTAGE DISTRIBUTION Very High	High-Medium	Low
Energy	28	72	0
Protein	78	22	0
Calcium	31	69	0
Zinc	60	40	0
Iron	24	76	0

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Energy Intake: According to the criteria used (refer to Section 5.3.5), about 28% of subjects had a very high energy intake, and the remainder had a high-medium energy intake. This was in accordance with the findings of the National Diet Survey (Birkbeck, 1979), which indicated that the "typical New Zealander" has a very high energy intake ie. higher for most age/sex categories than values reported from other developed countries.

Protein Intake: The frequent consumption of meat, bread and cereals and dairy products resulted in four-fifths of subjects having a very high protein intake, with the remainder having a high-medium protein intake. The National Diet Survey likewise indicated a very high intake of protein, especially animal protein.

Calcium Intake: Dairy products were consumed frequently so that one-third of subjects had a very high calcium intake; again there were no subjects in the low category. Generous calcium intakes, much of which came from milk or dairy products such as cheese were found in the National Diet Survey. The good calcium intake status of the present survey subjects could have been advantageous to them in terms of possible effects of a high cadmium intake. (Refer to Section 9).

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Zinc Intake: The frequent consumption of oysters in particular, and also of meat (especially beef), dairy products, bread and cereals resulted in more than half of subjects being classified as having a very high zinc intake. The remainder of subjects had a medium zinc intake. This survey population was distinct from most other New Zealanders because of their consumption of oysters, which distorted the pattern of dietary zinc intakes. In the National Diet Survey zinc intakes at the 90th centile were only marginally above the U.S. Recommended Daily Intake (RDA) for zinc (15 mg/d).

Iron Intake: One-quarter of subjects had a very high iron intake mainly because of their oyster and meat consumption. Again no subjects were categorised in the low intake group. As for calcium, such iron intakes would be advantageous in terms of potential effects of high cadmium intakes. A high intake of meat protein found in the National Diety Survey also resulted in high iron intakes (Birkbeck, 1979).

Overall, the survey subjects all appeared to consume generous - adequate intakes of the nutrients examined; no subjects were classified as having a low intake for any nutrient. This dietary data concorded with the usual dietary habits of New Zealanders, and with the rural nature of the area surrounding Bluff.

(c) Anthropometric Data (Appendix F)

Sex Distribution: The predominance of men in the oyster-fishing industry resulted in only 19 women participating in this study; 12 women participated in both the pre-season and the end-season sampling.

Age Distribution: For all subjects the mean age was 37 ± 12 (mean ± SD) years, with a range of 18-76 years. The women had a mean age of 34 ± 8 years, with a range of 18-48 years; the men had a mean age of 37 years, with a range of 18-76 years. Age distribution according to oyster intake categories (Table 18) was not greatly different; the youngest group was Category I and the oldest group was Category II.

Height and Weight Distribution: There was no difference in the height of subjects by category (Table 18), but body weight increased with increasing oyster intake. Category IV subjects on average weighed 9 kg more than did Category I subjects. Increasing body weight was not related to increasing age (Figure 9), as it was in the National Diet Survey, in which body weight increased by about 5 kg between subjects in their twenties and subjects in their forties. In the present study, however, a high proportion of the predominantly male subejcts was engaged in heavy physical acitvity so that this population sample was not typical of a randomly distributed "normal" population sample. The same caution as in the National Diet Survey needs to be used in interpreting the data in the present study because of its cross-sectional nature.

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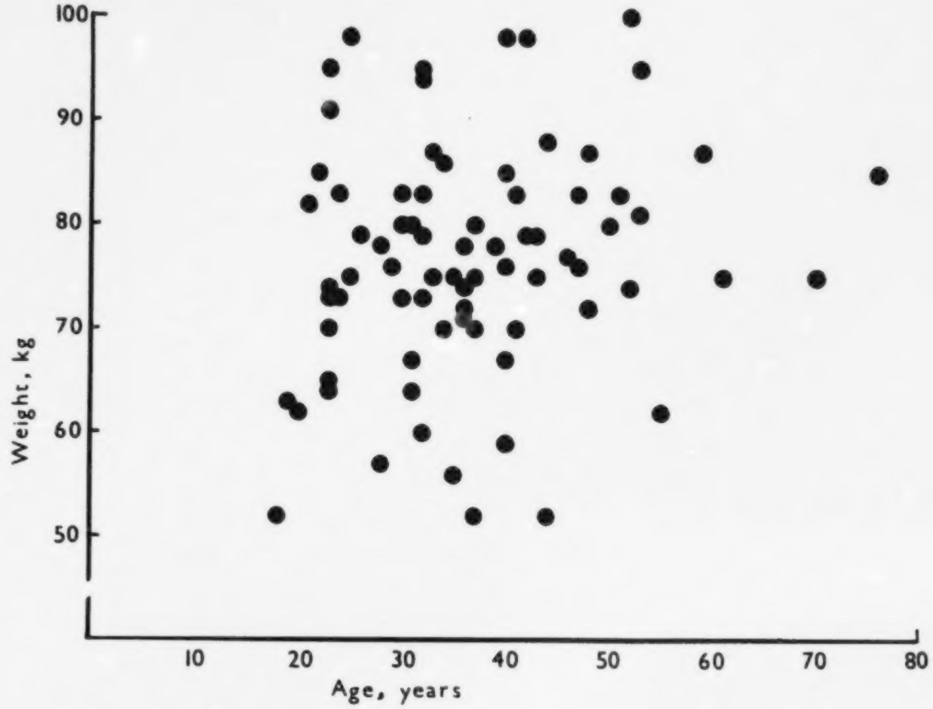

FIGURE 9: DISTRIBUTION OF BODY WEIGHT AGAINST AGE FOR ALL SUBJECTS

Quetelet's Index. This is defined as body mass index (BMI) where, BMI (Kg/m^2) = weight/$(height)^2$. This index of body size increased from Category I to Category IV (Table 18) because of the increased body weight of the subjects. Quetelet's Index for Category I subjects was 2.51 kg/m^2 and for Category IV subjects was 2.83 kg/m^2.

Smoking Habits: Subjects in Categories III and IV tended to smoke more cigarettes per day than did subjects in Categories I and II. On average Category IV subjects smoked the most (14 cigarettes/day).

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TABLE 18. SUMMARY OF ANTHROPOMETRIC DATA AND SMOKING HABITS ACCORDING TO OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	NUMBER OF SUBJECTS	SEX DISTRIBUTION	AGE yr	HT cm	WT kg	QUETELET'S INDEX kg/m^2	SMOKING CIGARETTES/d
I	18	F: 7 M: 11	31 ±11	170 ±11	74 ±7	2.51 ±0.38	9 ±13
II	23	F: 7 M: 16	40 ±12	169 ±12	75 ±13	2.64 ±0.38	6 ±9
III	23	F: 5 M: 19	36 ±10	168 ±9	80 ±9	2.80 ±0.31	11 ±14
IV	11	F: 0 M: 11	36 ±14	169 ±10	85 ±12	2.83 ±0.29	14 ±12

Values are mean ± SD.

6.2. Faecal Data

The dietary and faeces collection and analytical methods have been established in the Nutrition Department's laboratory for some years (Refer to Section 5). An earlier study (Guthrie & Robinson, 1977) found an average intake of 21 µg Cd/day in duplicate diets of young women. No population study of faecal cadmium outputs has been carried out but in metabolic balance studies cadmium intakes similar to those in the duplicate diets have been found.

6.2.1. Preliminary Study

As a part of the present study, a preliminary experiment was carried out. Faeces were collected from 2 female subjects for 2 days before and for 14 days after an oyster "feast" when 48 oysters were consumed by each person for breakfast. The "feast" gave calculated intakes of 1.58 mg cadmium, 16.6 mg zinc, 2.3 mg copper and 0.43 mg selenium. A Brilliant Blue faecal marker was ingested with the oysters; the marker appeared on the day following the "feast" for subject 1 and on the second day following the "feast" for subject 2. Subject 1 excreted an average of 10 µg Cd/day before the oyster "feast" (Figure 10), and a total of 1 mg Cd in the 14 days following, giving an apparent net "excretion" of 0.86 mg Cd from the oysters. Subject 2 excreted an average of 27 µg Cd/d before the oyster "feast" (Figure 10), and a total of 1.37 mg Cd in the following 14 days, giving an apparent net "excretion" of 1 mg cadmium from the oysters. For both subjects selenium excretion in the faeces (Figure 11) again resulted in apparent net "excretions" less than the calculated intakes. For subject 1 apparent net selenium "excretion" was 0.11 mg, and for subject 2 it was 0.16 mg.

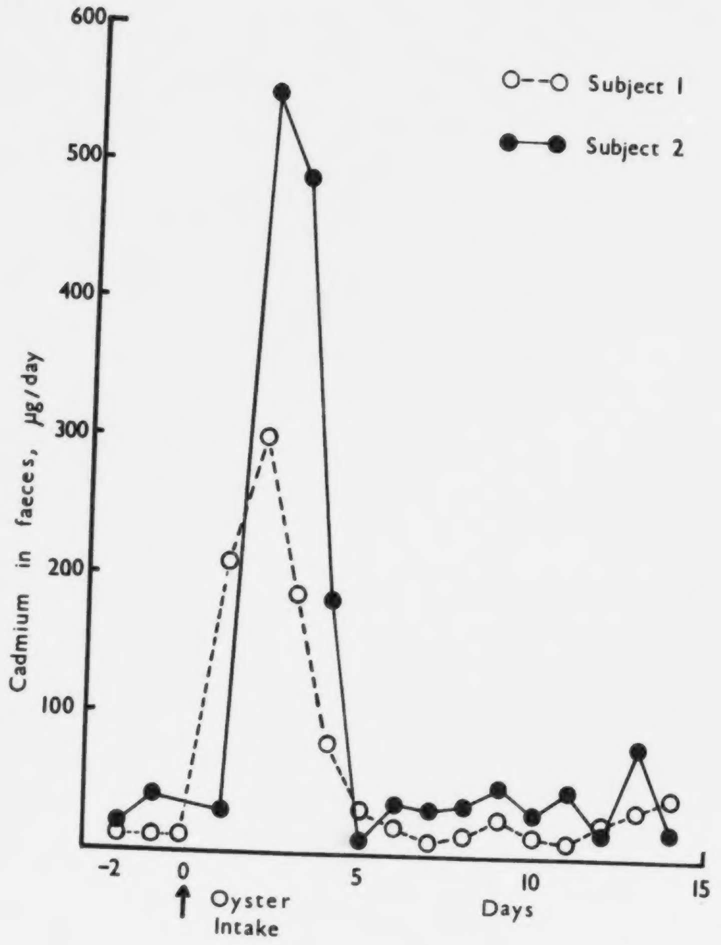

FIGURE 10: DAILY FAECAL EXCRETION OF CADMIUM FOR TWO SUBJECTS FOLLOWING OYSTER CONSUMPTION.

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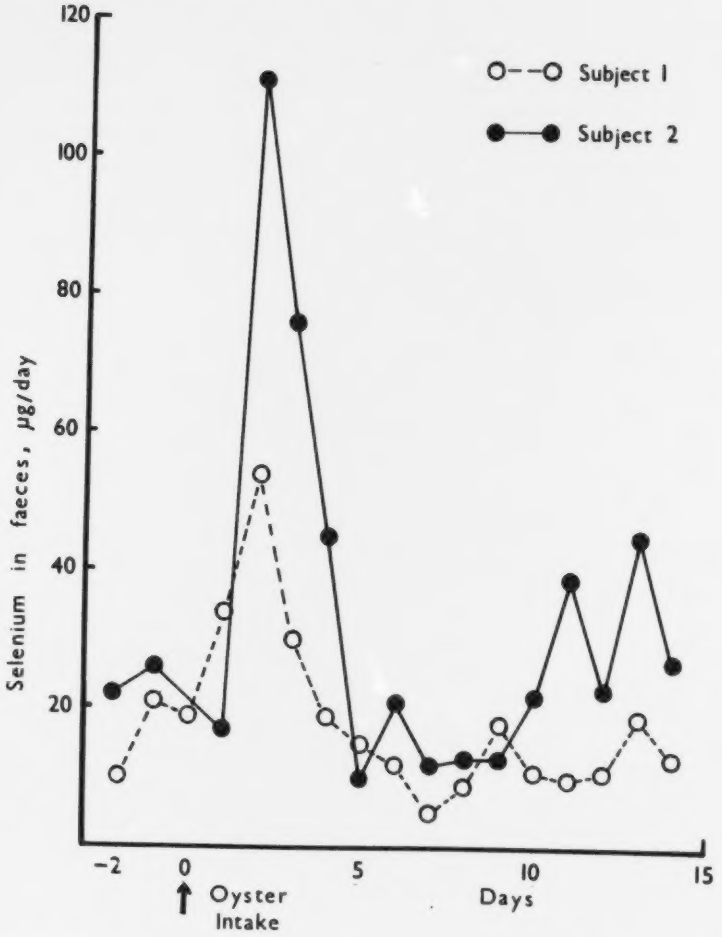

FIGURE 11: DAILY FAECAL EXCRETION OF SELENIUM FOR TWO SUBJECTS FOLLOWING OYSTER CONSUMPTION.

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6.2.2. Present Study

The individual results for the faecal analyses are given in Appendices G1-G8, according to oyster intake category.

(a) Pre-season Results: A summary of the pre-season results is given in Table 19. Weight of dried faeces eliminated over the three days of collections was greater for categories I and II than for categories III and IV; part of that greater faecal bulk was explained by the presence of a vegetarian in category I (dried faecal weight of 201 g) and also in category II (dried faecal weight of 139 g). Those two faecal weights were the greatest for the entire study (pre- and end-season) and were consistent with the known effect of vegetarian diets on faecal bulk.

TABLE 19. SUMMARY OF PRE-SEASON FAECES RESULTS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	NUMBER OF SUBJECTS	FAECES									
		Dry wt g/d	(n)	Cadmium µg/g	Cadmium µg/d	Zinc µg/g	Zinc mg/d	Copper µg/g	Copper mg/d	Manganese µg/g	Manganese mg/d
I	14	25 ±19	(10)	0.60 ±0.27	12 ±7	432 ±105	11 ±7	64 ±20	1.8 ±1.5	114 ±34	3.0 ±1.9
II	20	23 ±10	(14)	0.57 ±0.20	11 ±4	439 ±136	10 ±5	55 ±22	1.3 ±0.7	118 ±34	2.6 ±1.3
III	20	20 ±9	(15)	0.67 ±0.24	12 ±7	463 ±160	8 ±4	58 ±22	1.1 ±0.5	112 ±41	2.2 ±1.0
IV	6	17 ±8	(4)	0.70 ±0.42	12 ±8	447 ±85	8 ±4	51 ±9	0.9 ±0.5	125 ±32	2.2 ±1.4

Values are mean ± SD; concentrations are per g dried weight

Some subjects in each category were excluded from the cadmium calculations since they had already consumed some oysters, despite the season not having officially opened. Therefore the numbers of subjects represented in the cadmium data were 10 for category I; 14 for category II; 15 for category III and 4 for category IV. This was not necessary for zinc, copper and manganese because the contribution from oysters to the total daily intake of these elements was less than 3%. Neither the concentration of cadmium in faeces, nor the total output differed significantly among the categories. The output of the other elements was also very similar in the four groups.

The vegetarians higher than average intake of manganese influenced the group averages in groups I and II. Exclusion of the vegetarian subject in category I from the calculation of the output of manganese resulted in a mean of 2.8 ± 1.7 mg Mn/d; while exclusion of the vegetarian subject from category II resulted in a mean output of 2.5 ± 1.2 mg Mn/d.

The mean daily output of each element (derived by dividing the mean total output over 3 days by 3) is summarised in Table 20. It was considered valid to make comparisons of daily faecal outputs on a group basis because the vagaries of intermittent faecal elimination over a three-day period would have been evened out for the group. The daily faecal excretions of each element appeared reasonable and consistent with the data on daily intakes of these elements by normal New Zealand adults, i.e. 15 µg cadmium, 10 mg zinc, 1.5 mg copper and 2.7 mg manganese (Table 4). Since the faeces is the main route of excretion of zinc, copper and manganese, and probably also of cadmium, it can be expected that daily faecal output (which will represent both exogenous and endogenous losses) will be similar to daily intake, assuming no recent change in dietary habits.

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TABLE 20. MEAN DAILY PRE-SEASON OUTPUT OF CADMIUM, ZINC, COPPER AND MANGANESE FOR EACH OYSTER INTAKE CATEGORY

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OYSTER INTAKE CATEGORY	DAILY FAECAL OUTPUT Cadmium µg/g	Zinc mg/g	Copper mg/g	Manganese mg/g
I	12	11	1.8	3.0
II	11	10	1.3	2.6
III	12	8	1.1	2.2
IV	12	8	0.9	2.2

(b) End-Season Results: A summary of the end-season results is given in Table 21. Weight of dried faeces again showed a trend towards a lower amount for categories III and IV, but since there were now no vegetarians in any group, the difference among the groups was not as great. Concentrations of cadmium in dried faeces increased with an increasing oyster intake, so that the mean for category IV was 12½ times greater than the mean for category I. For category IV the end-season concentration (Table 21) was 18 times higher than the pre-season concentration (Table 19). These increases were statistically significant (t-test, $p < 0.05$). In categories II and III the end-season cadmium concentrations were also significantly increased. Total excretion of cadmium over the 3 days also increased with an

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increasing oyster intake; for all categories except category I there was a large variation among subjects in their total cadmium excretion, reflecting varying periods of time since oysters had last been eaten. For category IV the variation was especially wide: 36-1503 µg Cd/3d. The highest individual excretion of cadmium over the 3 days of faecal collections was 1739 µg Cd for a category II subject.

TABLE 21. SUMMARY OF END-SEASON FAECES RESULTS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	NUMBER OF SUBJECTS	FAECES								
		Dry wt	Cadmium		Zinc		Copper		Manganese	
		g/d	µg/g	µg/d	µg/g	mg/d	µg/g	mg/d	µg/g	mg/d
I	9	22 ±20	0.99 ±0.83	15 ±8	435 ±86	9 ±8	60 ±17	1.1 ±0.9	132 ±40	3.0 ±3.5
II	19	21 ±11	3.96 ±5.00	84 ±134	514 ±140	11 ±6	68 ±21	1.4 -0.7	132 ±43	2.8 ±1.7
III	19	17 ±8	8.97 ±9.87	129 ±144	632 ±338	10 ±6	76 ±27	1.3 ±0.6	112 ±37	1.9 ±0.8
IV	7	18 ±8	12.4 ±10.2	233 ±185	633 ±110	12 ±6	69 ±7	1.2 ±0.5	124 ±103	2.3 ±0.9

Values are mean ± SD; concentrations are per g dried weight

Concentration and total excretion of zinc increased slightly with an increasing oyster intake, whereas there was no difference for copper. Output of manganese again tended to decrease from category I to category IV, but oyster intake was not expected to influence faecal excretion of manganese because of the minor impact of oysters on daily manganese intake (Table 4).

The daily output of each element is summarised in Table 22; the influence of oyster consumption on daily cadmium excretion resulted in a 15½ times increase in daily faecal output of cadmium (category I compared with category IV). For zinc, category IV excreted 1/3 more than did category I.

Figure 12 shows the frequency distribution of daily faecal cadmium excretion for each intake category; the wide variation among subjects in categories II, III and IV was evident. Since the division into oyster intake categories was based on the average for the season, recent oyster intake would not necessarily have been similar to that average consumption.

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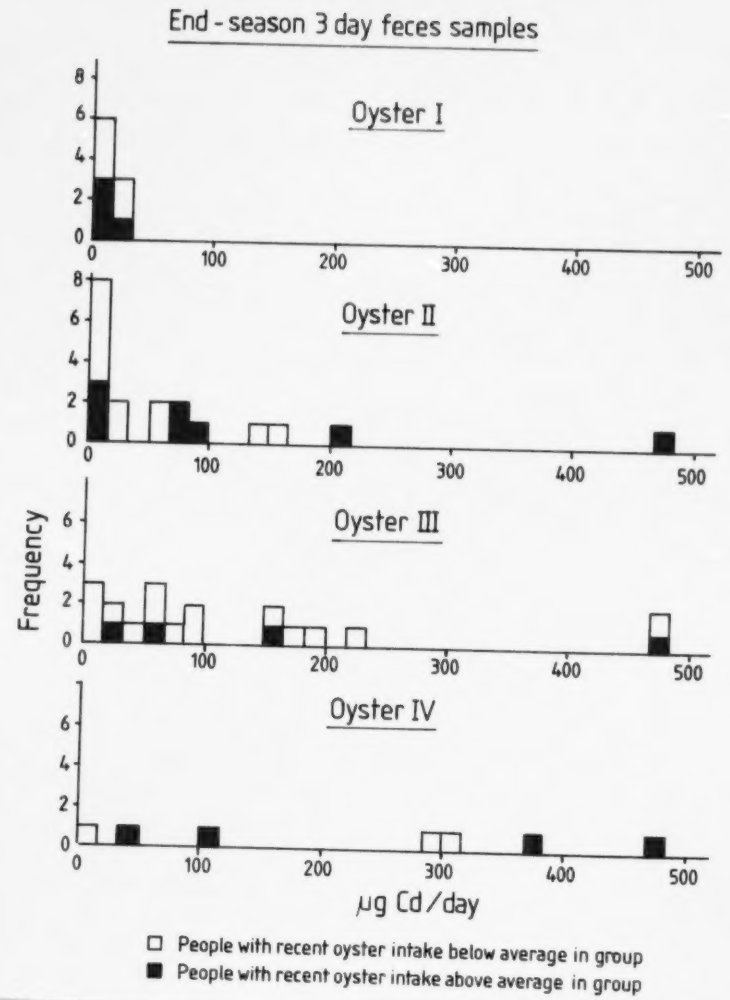

FIGURE 12: FREQUENCY DISTRIBUTIONS OF DAILY FAECAL CADMIUM EXCRETION IN THE END-SEASON FAECAL SAMPLES FOR EACH OYSTER INTAKE CATEGORY.

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TABLE 22. MEAN DAILY END-SEASON OUTPUT OF CADMIUM, ZINC COPPER AND MANGANESE FOR EACH OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	DAILY FAECAL OUTPUT			
	Cadmium µg/d	Zinc mg/d	Copper mg/d	Manganese mg/d
I	15	9	1.1	3.0
II	84	11	1.4	2.8
III	129	10	1.3	1.9
IV	233	12	1.2	2.3

The average intake of oysters over the season was greater than it was at the end of the season (refer to Table 16). Since the end-season oyster intake was known, cadmium excretion in faeces could be compared to the recent intakes. Figure 13 presents the individual results for daily end-season faecal cadmium excretion against end-season oyster intake; despite the wide individual variation among subjects, the group mean output of cadmium was closely related to oyster intake over the last 3 days ($r = 0.892$) in the four intake categories. The regression equation for the 4 mean cadmium excretions against recent oyster intake was Faecal cadmium = 23.3 x oysters + 14.9 (µg/d); Figure 14 presents the regression (Cd observed), compared with an "expected" regression calculated assessing a background cadmium intake of 15 µg/d and a cadmium content of 24 µg/oyster (Table 4). There was a remarkable agreement between the "observed" and the "expected" regressions, indicating that on a group basis faecal cadmium output closely represented the cadmium intake from oysters.

Calculation of "observed" and "expected" regressions for zinc again gave a close agreement (Figure 14), even though the relative increase of zinc intake from oysters (Table 4) was not great compared to the large relative increase in cadmium intake. Thus again on a group basis faecal output of zinc reflected zinc intake from oysters.

The contribution from oysters to the total daily intake of copper and manganese was less than 3% (Table 4) and hence was not expected to have any significant effect on the faecal excretion of these metals. It is clear from the observed data (Figure 15) that this is true for copper, although the group average faecal copper values were slightly lower than that expected. The observed apparent decrease in faecal manganese with the increase in oyster intake was unexpected. It is possible that factors such as variable dietary manganese intake and

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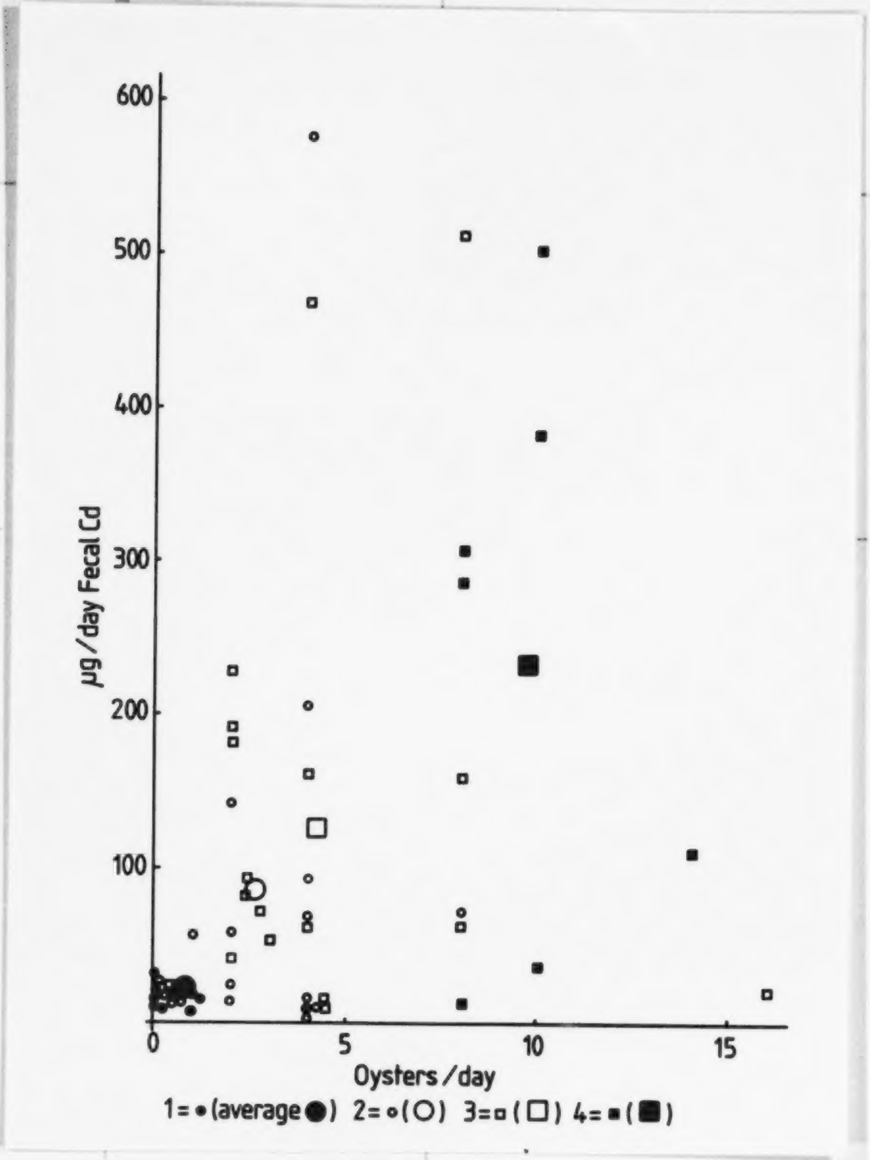

FIGURE 13: DAILY FAECAL CADMIUM EXCRETION IN THE END-SEASON FAECAL SAMPLES RELATED TO END-SEASON OYSTER INTAKE. SMALL SYMBOLS REPRESENT AN INDIVIDUAL RESULT, WHILE LARGE SYMBOLS REPRESENT THE MEAN RESULT FOR AN OYSTER INTAKE CATEGORY: ● = Category I; ○ = Category II; ▫ = Category III; ■ = Category IV.

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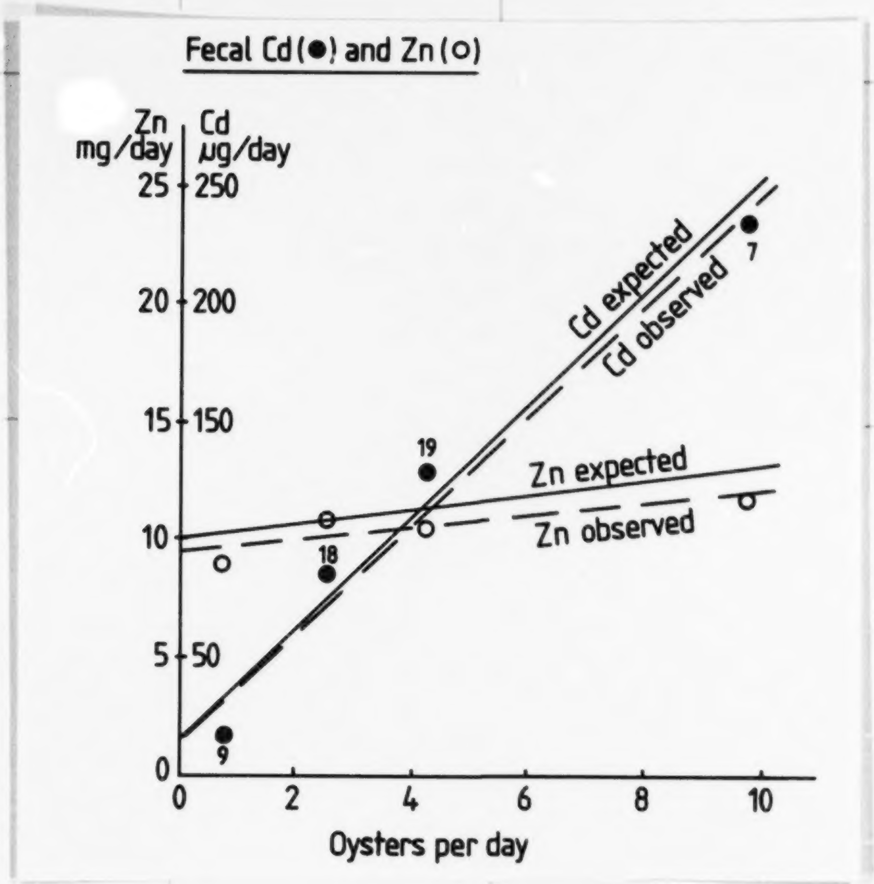

FIGURE 14: REGRESSIONS OF "OBSERVED" AND "EXPECTED" END-SEASON FAECAL EXCRETION OF CADMIUM AND ZINC AGAINST END-SEASON OYSTER INTAKE. SEE TEXT FOR EXPLANATION OF "EXPECTED" REGRESSIONS.

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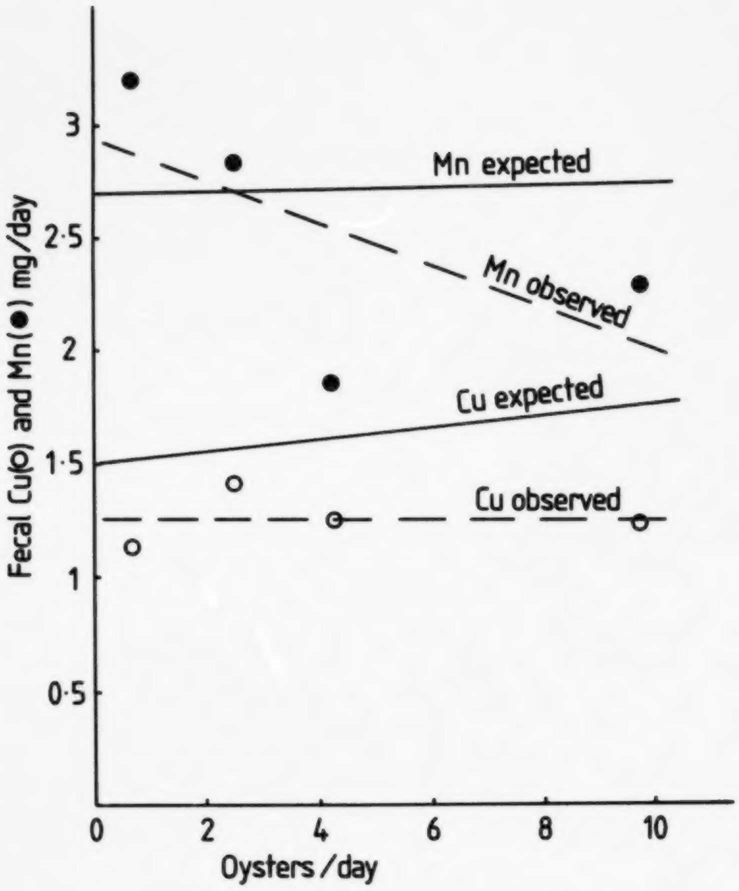

FIGURE 15: REGRESSION OF "OBSERVED" AND "EXPECTED" END-SEASON FAECAL EXCRETION OF COPPER AND MANGANESE AGAINST END-SEASON OYSTER INTAKE. SEE TEXT FOR EXPLANATION OF "EXPECTED"REGRESSIONS.

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trace element interactions may have influenced the observed results.

The other trace element investigated in this present study was selenium. Each oyster would have increased the daily selenium intake by about 10% (Table 4). Faecal excretion of selenium was not analysed in this study, but as seen from the blood selenium concentrations (Section 6.3.2.) there was reason to believe that selenium intake had increased. Therefore selenium excretion in the faeces probably increased also, as it did in the preliminary study (Figure 11).

The daily faecal excretion of cadmium was calculated for those subjects in the four categories who gave samples both pre-season and at the end of the season (Table 23). Again, subjects who had recently consumed oysters were excluded from the pre-season calculations. The results for these subjects were essentially the same as for the total number of subjects who participated at each sampling i.e. no difference in cadmium excretion among the groups before the season commenced (Table 20), and about a 15-fold increase from category I to category IV at the end of the season (Table 23). The increase during the season in cadmium excretion was 9-fold for both category II and category III.

TABLE 23. DAILY FAECAL EXCRETION OF CADMIUM FOR THOSE SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	NUMBER OF SUBJECTS	DAILY FAECAL OUTPUT OF CADMIUM Pre-Season µg/d	DAILY FAECAL OUTPUT OF CADMIUM End-Season µg/d
I	3	9	10
II	11	11	103
III	11	12	113
IV	3	12	143

6.2.3. Comparison of Present Study with Japanese Studies.

It was of interest to compare the faecal excretions of the trace elements considered in the present study with the results from other studies. One such Japanese study (Tsuchiya & Iwao, 1978) analysed cadmium, zinc, copper and lead in five-day samples of faeces. A polluted area, a rural control area and a group of Tokyo inhabitants were compared (Table 24) and it was found that the excretions of both zinc and copper were increased along with cadmium excretion in the polluted areas.

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TABLE 24. DAILY EXCRETIONS OF CADMIUM, ZINC, COPPER AND LEAD IN A POLLUTED AREA, A RURAL CONTROL AREA AND IN TOKYO INHABITANTS IN JAPAN*

AREA	NUMBER OF SUBJECTS	FAECAL EXCRETION (FROM 5-d SAMPLES), MEAN ± SD			
		Cadmium µg/d	Zinc mg/d	Copper mg/d	Lead µg/d
Polluted area	30	198 ±114	21.6 ±12.5	2.71 ±1.82	226 ±206
Rural control area	30	48.6 ±41.9	10.3 ± 4.7	1.27 ±0.73	36.3 ±32.5
Tokyo inhabitants	19	36.2 ±31.2	10.5 ± 6.5	1.32 ±0.91	45.0 ±26.0
Difference between polluted and rural control area, µg/d		149	11300	1440	190

*From: Tsuchiya & Iwao, 1978

In another study (Matsuo & Kodama, 1981) the cadmium, zinc, copper and manganese concentrations in polluted rice as well as non-polluted rice were analysed by direct flame atomic absorption (Table 25). From that data the contribution of different elements to the daily intake was calculated assuming that 300 g cadmium-polluted rice or non-polluted rice was consumed (Table 26). It was interesting to note (Table 27) that the increase in zinc and copper intakes if 100 µg Cd were consumed from either Bluff oysters or from Japanese polluted rice would be very similar whereas the manganese intake would be 10 times higher from the rice than from the oysters. On the other hand, in the study of cadmium in faeces the actual increases in zinc and copper intakes were much greater than the increases expected from the analysis of the Japanese polluted rice. The rice analysed might not necessarily have been from the same area as was studied for faecal cadmium and there might be great variations in the distributions of trace elements in different types of polluted rice.

In any case, the calculations indicated that the oysters may contribute less to the intake of some essential trace elements than would Japanese rice from polluted areas. Unfortunately selenium was not analysed in the food, but since the selenium concentration in cereals in general is higher than in other foods (except in New Zealand where the soils have a low selenium content) it seemed possible that the

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total selenium intake also would have increased because of the contribution from Japanese rice as was true for the oysters. A discussion of possible interactions among the elements is found in section 9.

TABLE 25. CADMIUM, ZINC, COPPER AND MANGANESE CONTENT IN POLLUTED AND NON-POLLUTED JAPANESE RICE*

RICE	NUMBER OF SAMPLES	CONCENTRATION, µg/g Cadmium	Zinc	Copper	Manganese
Cd-polluted rice	7	1.2	29.6	5.09	27.9
Non-polluted rice	9	0.04	16.3	3.68	26.2

*from: Matsuno & Kodama, 1981

TABLE 26. CALCULATED CONTRIBUTION OF CADMIUM-POLLUTED OR NON-POLLUTED RICE ASSUMING AN INTAKE OF 300 g RICE (CALCULATED FROM THE DATA IN TABLE 25)

	CONTRIBUTION, µg Cadmium	Zinc	Copper	Manganese
Cd-polluted rice	360	8880	1527	8370
Non-polluted rice	12	4890	1104	7860
Increase	348	3990	423	510

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TABLE 27. COMPARISON OF CALCULATED INCREASES (µg/d, % OF DAILY INTAKE) IN INTAKE OF ZINC, COPPER AND MANGANESE ASSOCIATED WITH AN INTAKE OF 100 µg CADMIUM EITHER FROM BLUFF OYSTERS OR FROM JAPANESE POLLUTED RICE

CADMIUM SOURCE	INCREASE IN INTAKE *							
	Cadmium		Zinc		Copper		Manganese	
	µg/d	%	µg/d	%	µg/d	%	µg/d	%
Bluff oysters	100	667	1333	13.3	108	7.2	17.5	0.6
Japanese polluted rice (Table 26)	100	667	1147	11.5	122	8.1	147	5.4
Food(mainly Rice) in a Japanese polluted area (Table 24)	100	667	7584	75.8	966	64.4	147	5.4

* % increase calculated in relation to "normal" daily intake in New Zealand (Table 4).

6.3. Whole Blood and Serum Data

6.3.1. Haemoglobin concentration and Packed Cell Volume

The individual results for haemoglobin concentration and for packed cell volume pre-season and end-season for each category are presented in Appendices H1-H4. Table 28 summarises those results for men and for women in each oyster intake category. Within each category men consistently had the expected higher haemoglobin concentration and packed cell volume, and this was true throughout the season. For each category there was no difference in haemoglobin concentrations nor in packed cell volumes at the end compared with the beginning of the season. Thus there had been no apparent change in iron status as measured by these two paramaters which were within normal limits for the men and for the women, respectively.

6.3.2. Whole Blood Cadmium Concentration

The individual results for whole blood cadmium concentration and for the other trace elements measured on the blood samples are presented in Appendices I1-I4.

TABLE 28. SUMMARY OF HAEMOGLOBIN CONCENTRATION AND PACKED CELL VOLUME FOR MEN AND FOR WOMEN BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	SEX	PRE-SEASON Number of subjects	PRE-SEASON Hb g/100 ml	PRE-SEASON PCV %	END-SEASON Number of subjects	END-SEASON Hb g/100 ml	END-SEASON PCV %
I	M	10	15.8 ± 1.2	45 ± 2	9	15.2 ± 0.9	45 ± 2
	F	5	14.0 ± 0.8	42 ± 3	3	12.3 ± 1.2	37 ± 2
II	M	13	15.5 ± 1.3	44 ± 2	12	15.3 ± 1.1	44 ± 2
	F	7	14.0 ± 1.7	41 ± 2	6	14.0 ± 1.6	42 ± 4
III	M	16	15.4 ± 1.9	44 ± 3	16	15.5 ± 1.2	44 ± 3
	F	5	14.3 ± 2.1	41 ± 4	4	14.4 ± 0.3	42 ± 4
IV	M	7	15.5 ± 1.6	44 ± 5	8	15.7 ± 1.1	45 ± 3

Values are mean ± SD.

The mean cadmium concentration in the blood of 17 Dunedin controls who were not regular oyster consumers and who had not eaten any oysters in the four weeks prior to sampling was 0.90 ± 0.28 ng/ml (Table 29); none of these controls were smokers. This control concentration was comparable with that reported for unexposed workers in Sweden (0.5 ng/g for non-smokers and 2.0 ng/g for smokers; Kjellstrom, 1979). The oyster consumers had overall mean whole blood cadmium concentrations significantly higher ($P<0.01$) than the control group both pre-season (3.12 ng/ml) and end-season (4.0 ng/ml). These concentrations were similar to those reported for unexposed workers in the United States (3.8 ng/ml; Baker et al., 1979) and in Japan (4.5 ng/g; Harada et al., 1974).

For each oyster category the whole blood cadmium concentrations pre-season compared with end-season are presented in Table 30. Although for the faecal data some subjects had to be excluded from the calculation of the mean pre-season concentration because of their recent consumption of oysters, this did not appear to be necessary for the calculation of the pre-season blood data (because exclusion of the blood data for those subjects

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TABLE 29. WHOLE BLOOD CADMIUM CONCENTRATION IN THE DUNEDIN CONTROL GROUP

CONTROL SUBJECT NUMBER	WHOLE BLOOD CADMIUM ng/ml
1	1.01
2	1.36
3	1.33
4	0.93
5	0.57
6	0.68
7	1.16
8	0.81
9	1.01
10	0.91
11	0.87
12	0.53
13	0.85
14	0.59
15	1.20
16	0.41
17	1.04
n	17
$\bar{x}$	0.90
SD	0.28

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TABLE 30. MEAN WHOLE BLOOD CADMIUM CONCENTRATIONS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	WHOLE BLOOD CADMIUM, ng/ml * PRE-SEASON Number	$\bar{x}$ ± SD	END-SEASON Number	$\bar{x}$ ± SD
I	15	2.58±1.87	12	3.45±2.12
II	19	2.90±2.33	18	2.87±1.84
III	21	3.53±2.32	20	4.40±2.82
IV	7	3.72±2.73	8	5.85±2.15

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made no difference to the means). Cadmium in blood tended to increase with increasing oyster consumption even for the pre-season results, and the end-season means appeared higher than the pre-season means for all except Category II. Because of the great individual variations, these trends were not statistically significant.

However because of the known effect of smoking on blood cadmium the data was recalculated to determine the effect of smoking within each category of oyster consumption, for both the pre-season and the end-season results (Table 31). For the non-smokers a trend towards a higher blood cadmium with an increasing oyster consumption remained, even for the pre-season data. At the end of the season the mean concentration had risen by 0.3-0.6 ng/ml in each category. The smokers had a mean blood cadmium consistently above that for the non-smokers both pre-season and especially end-season. The trend towards a higher mean concentration from Category I to Category IV was not as obvious pre-season, but at the end of the season the combined effect of smoking and eating oysters gave Category IV smokers the highest mean concentration of 6.70± 1.75 ng/ml. Even Category I smokers

TABLE 31. WHOLE BLOOD CADMIUM CONCENTRATION FOR NON-SMOKERS COMPARED WITH SMOKERS BY OYSTER INTAKE CATEGORY, AND FOR PRE-SEASON COMPARED WITH END-SEASON DATA

OYSTER INTAKE CATEGORY	WHOLE BLOOD CADMIUM CONCENTRATION, ng/ml * PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
		A. NON-SMOKERS		
I	10	1.69±0.98	7	2.12±1.28
II	14	1.74±1.03	13	2.08±1.05
III	10	2.08±0.95	10	2.63±1.01
IV	2	2.70(±0.65)	2	3.35(±0.50)
		B. SMOKERS +		
I	5	4.35±2.06	5	5.40±0.97
II	6	5.60±2.32	5	4.91±1.97
III	10	5.07±2.46	10	6.20±2.99
IV	5	4.12±3.21	6	6.70±1.75

+ The blood cadmuim values for smokers were significantly higher than those for the corresponding non-smoker group (Student's t-test, $p<0.05$).

had a higher mean blood cadmium at the end of the season compared with pre-season. For all except Category II subjects the mean increase was greater than 1 ng/ml at the end of the season, i.e. an increase about twice as great as for the non-smokers. The end-season mean blood cadmiums for the smokers were more than double those for the non-smokers.

For all subjects (smokers and non-smokers) the correlation between their pre-season blood cadmium and their end-season blood cadmium was good (r=0.93). As Figure 16 indicates the end-season value was higher than the pre-season value for the majority of subjects.

For those subjects who participated both at the beginning and at the end of the season the blood cadmium data is presented in Table 32 for non-smokers and for smokers. Although the number of subjects in each category was fewer the data is similar to that in Table 31 except that for the smokers the end-season mean values were not different from the pre-season mean values for Categories I, II and IV; however the number of subjects in these three categories was small. Within each category blood cadmium levels were significantly higher (student's t-test, $p<0.05$) for smokers than for non-smokers.

TABLE 32. WHOLE BLOOD CADMIUM CONCENTRATION FOR NON-SMOKERS AND FOR SMOKERS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	WHOLE BLOOD CADMIUM CONCENTRATION, ng/ml* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
		A. NON-SMOKERS		
I	6	1.75±1.22	6	2.24±1.36
II	11	1.72±1.16	11	1.96±0.91
III	8	1.84±0.72	8	2.84±0.98
IV	1	(3.16)	1	(3.70)
		B. SMOKERS +		
I	3	5.73±1.14	3	5.71±1.12
II	5	4.99±1.98	5	4.91±1.97
III	10	5.07±2.46	10	6.20±2.99
IV	3	6.15±2.29	3	6.24±1.01

+As for Table 31

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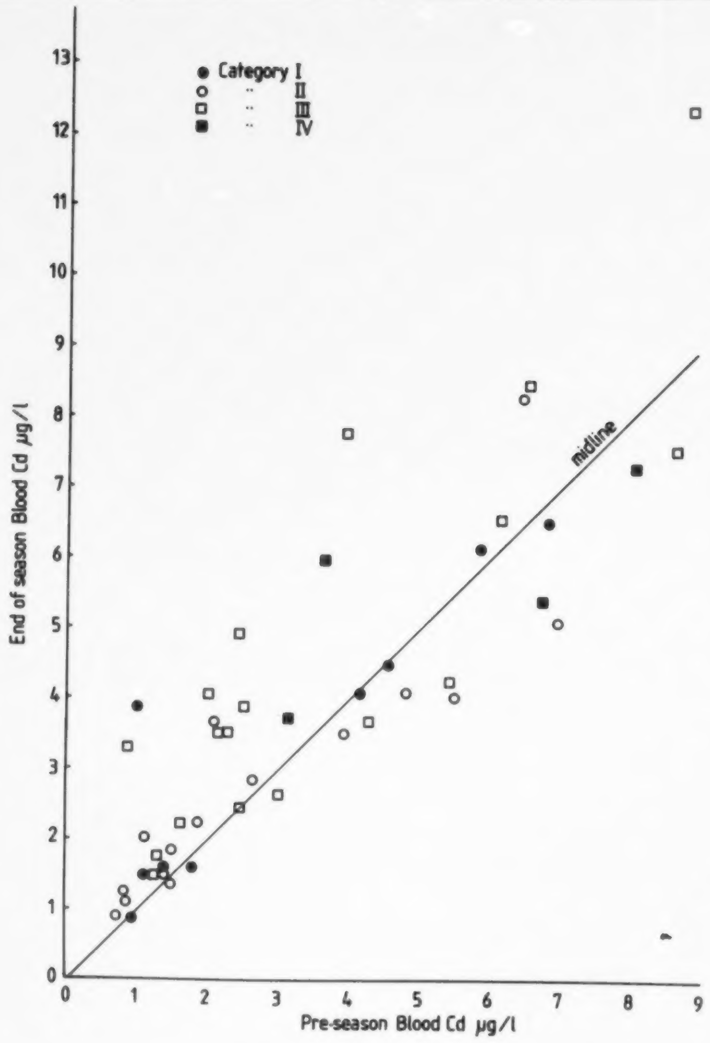

FIGURE 16: PRE-SEASON WHOLE BLOOD CADMIUM CONCENTRATION COMPARED WITH END-SEASON WHOLE BLOOD CADMIUM CONCENTRATION FOR SUBJECTS BY OYSTER INTAKE CATEGORY: ● = Category I; O = Category II; □ = Category III; ■ = Category IV.

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6.3.3. Whole Blood Selenium Concentration

Appendices I1-I4 contain the individual data on selenium which is summarised in Table 33. As for cadmium there was a trend for the mean values to increase with an increased consumption of oysters, but this was particularly evident in the end-season mean values for Category III and Category IV subjects. The additional selenium intake from oysters in relation to the usual daily intake (Table 4, Table 16) was not sufficient to have enhanced the blood selenium concentration for Category I or Category II subjects. However for Category III and IV subjects their higher seasonal oyster intakes resulted in an additional 14.6 µg and 51.0 µg Se/day, respectively. Since for Category IV subjects this additional selenium intake would more than have doubled their usual intake it was not surprising that their blood selenium values had increased.

TABLE 33. SUMMARY OF WHOLE BLOOD SELENIUM CONCENTRATION BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	WHOLE BLOOD SELENIUM CONCENTRATION, ng/ml* PRE-SEASON		END-SEASON	
	Number of subjects	$\bar{x} \pm$SD	Number of subjects	$\bar{x} \pm$ SD
I	15	65±19	12	69±19
II	20	70±14	18	71±19
III	21	72±13	20	87±17
IV	7	79± 8	7	100±19

Most subjects also ate fish or other shellfish occasionally and this would also have increased their usual selenium intake; Category IV subjects did not eat more fish than did the other subjects however.

Smoking had no influence on blood selenium concentration.

For those subjects who participated at both sampling times the same pre-season trend in mean values was noted (Table 34), and again for Category III and Category IV subjects the end-season mean values were notably higher in comparison both with Category I and II subjects and with their own pre-season values. Category IV subject 14.4 TB had an unusually high end-season value of 174 ng Se/ml; if this result is considered as an outlier and excluded the average pre-season and end-season concentrations would be 83 and 107 ng Se/ml respectively.

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TABLE 34. SUMMARY OF WHOLE BLOOD SELENIUM CONCENTRATION FOR SUBJECTS WHO PARTICIPATED BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	WHOLE BLOOD SELENIUM CONCENTRATION, ng/ml* PRE-SEASON Number of subjects	x̄ ± SD	END-SEASON Number of subjects	x̄ ± SD
I	9	65±21	9	69±20
II	16	67±12	16	71±19
III	18	74±13	18	87±17
IV	3	83±10	3	107±17

6.3.4. Serum Zinc and Copper Concentration

Appendices I1-I4 contain the individual data for serum zinc and serum copper concentrations.

TABLE 35. SUMMARY OF SERUM ZINC CONCENTRATIONS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	SERUM ZINC CONCENTRATION, μg/100 ml* PRE-SEASON Number of subjects	x̄ ± SD	END-SEASON Number of subjects	x̄ ± SD
I	6	69± 8	13	99±20
II	14	61±10	18	74±17
III	19	61±13	20	78±15
IV	5	60±13	7	77±18

Any obviously haemolysed samples were not included in the serum zinc analysis because of possible zinc contamination from the red blood cells. There was no trend in serum zinc concentrations in the pre-season mean values (Table 35), which were at the lower end of a normal concentration range for healthy adults (McKenzie, 1979). End-season mean values were between

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13 and 30 μg Zn/100 ml higher than the pre-season values, with the greatest increase being for Category I subjects.

For those subjects who gave samples at both times, the end-season values were not significantly higher than the pre-season values, nor was there a difference among the categories at the beginning or the end of the season. These results confirmed the independent nature of serum zinc concentration in relation to variations in dietary zinc intake (McKenzie, 1979). Even though for Category IV subjects their increased intake of zinc from oysters (Table 4) using their seasonal average intake (Table 16) would have been 5.8 mg Zn i.e. about a 50% enhancement of their usual dietary intake (Table 4) they did not have a higher end-season serum zinc concentration than did the other categories.

TABLE 36. SUMMARY OF SERUM ZINC CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	SERUM ZINC CONCENTRATION, μg/100 ml* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
I	5	68±8	5	82±14
II	11	59±7	11	71±18
III	16	62±13	16	77±13
IV	4	57±12	4	69±10

The summary of values for serum copper concentration by oyster intake category is presented in Table 37. Women who were known to be on an oral contraceptive, or pregnant, were excluded from the calculation of the mean values. Pre-season mean values did not differ among the categories, and neither did the end-season mean values differ among the categories. However the end-season values all were lower than the pre-season values, and this difference persisted for those subjects who gave samples at both times (Table 38). Since all the sera were analysed at the same time using the same standard solutions it was considered unlikely that this was a methodological difference. Since the copper concentrations in all groups were reduced to about the same extent it was considered unlikely also that this difference could have been attributed to eating oysters. It was interesting to note that the zinc and copper values showed a reverse trend (Tables 36 and 38). Copper and zinc are mutually antagonistic but whether there was a competitive mechanism operating in these subjects is not known. Season variations in serum copper concentration have not been reported earlier.

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TABLE 37. SUMMARY OF SERUM COPPER CONCENTRATIONS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	SERUM COPPER CONCENTRATION, µg/100 ml* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
I	6	120±26	13	99±20
II	14	125±28	19	99±23
III	17	113±17	19	96±21
IV	5	127±19	7	97±23

TABLE 38. SUMMARY OF SERUM COPPER CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

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OYSTER INTAKE CATEGORY	SERUM COPPER CONCENTRATION, µg/100 ml* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
I	4	129±27	4	98±13
II	11	120±27	11	93±19
III	16	111±22	11	100±30
IV	4	121±16	4	96±26

6.3.5. Serum beta-2-microglobulin Concentration

The individual data are presented in Appendices I1-I4 and are summarised in Table 39. Only the end-season sera were analysed for beta-2-microglobulin concentration. There was no difference in mean serum beta-2-microglobulin concentration among the categories, and the values were considered to be within the normal range for healthy adults (Wibell, 1976).

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TABLE 39. SUMMARY OF END-SEASON SERUM BETA-2-MICROGLOBULIN CONCENTRATION BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	SERUM BETA-2-MICROGLOBULIN CONCENTRATION, mg/l Number of subjects	$\bar{x} \pm$ SD
I	12	2.2±0.5
II	17	2.2±0.6
III	20	2.1±0.3
IV	8	2.4±0.7

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6.4. Urine Data

6.4.1. Specific Gravity, pH, Creatinine, Urea, Glucose and Protein

Appendices J1-J4 present the individual data for this baseline information for subjects in Category I - Category IV. The range in the specific gravity of these overnight urine samples both pre-season (1.010-1.037) and end-season (1.010-1.039) was similar to the range in urinary specific gravity for normal adults. Similarly the range in pH both pre-season (pH5-pH7) and end-season (pH4.7-pH6.9) was similar to the range in urinary pH for normal adults consuming freely chosen diets. No elevated levels of glucose was detected in any pre-season urines or in any end-season urines. Marginally elevated protein concentrations were detected in 11 pre-season urines, and in 17 end-season urines. Only one subject (25.2.LC) who had previously been treated for a urinary tract infection, showed any significant urinary protein level. The incidence of traces of protein within oyster intake categories for end-season urines was 18% (2/11) for Category I, 37% (7/19) for Category II, 20% (4/20) for Category III and 43% (3/7)for Category IV. Creatinine and urea concentrations seemed to be appropriate for overnight specimens varying several-fold in volume from subject to subject.

6.4.2. Urinary Cadmium Concentration

The same Dunedin people who acted as controls in providing a blood sample (Table 29) also provided an overnight urine sample, and the results are presented in Table 40. The mean cadmium concentration for 16 subjects was 0.62±0.34 ng/ml, with a range of 0.17-1.25. These concentrations were comparable with those reported for non-smoking adults from the U.S.A.. Kjellstrom (1979) reported that in Sweden, the U.S.A. and Japan the mean cadmium concentration in the urine of non-smoking adults was 0.3, 0.67 and 1.27 ng/ml, respectively.

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TABLE 40. URINARY CADMIUM CONCENTRATION IN CONTROL GROUP

CONTROL SUBJECT SUBJECT	URINARY CADMIUM ng/ml
1	0.34
2	0.96
3	0.34
4	0.28
5	0.45
6	0.17
7	1.25
8	0.68
9	1.08
10	0.51
11	1.14
12	0.50
13	0.36
14	0.55
15	0.36
16	0.91
n	16
$\bar{x}$	0.62
SD	0.34

Appendices K1-K4 present the individual data for urinary cadmium concentration expressed both as ng/ml and as molar ratio with creatinine (μmol/mol). Table 41 summarises the urinary cadmium data by oyster intake category for all subjects, and shows that all of the mean values were about three times greater than for the control subjects (Table 40). There was no consistent trend in urinary cadmuim concentration expressed either as a concentration (ng/ml) without taking account of the extent of dilution of the urine, or as a molar ratio with creatinine (μmol/mol) i.e. making some correction for the extent of dilution of the urine. Category IV subjects, who might have been expected to have excreted more cadmium in their urine if their higher intake had resulted in an accumulation of cadmium in their renal tissue had a lower cadmium concentration than did Category III (and Category I) subjects both pre-season and at the end of the season. Furthermore, there was not an increased urinary cadmium concentration within any oyster intake category when the end-season data were compared with the pre-season data.

As indicated from Figure 17 there was a large individual scatter in the urinary cadmium concentration (ng/ml) and no significant age trend was found. Other studies (Kjellstrom, 1979) have shown a relationship between urinary cadmium and age with lower levels at young and old ages than in the middle age group. In the present study most of the subjects were within the age range 20-50 years and the group average levels (Table 41) were about twice as high as those previously reported for this age group (Kjellstrom, 1979).

TABLE 41. SUMMARY OF URINARY CADMIUM CONCENTRATION BY OYSTER INTAKE CATEGORY*

OYSTER INTAKE CATEGORY	PRE-SEASON Number of subjects	PRE-SEASON Urinary cadmium concentration ng/ml	PRE-SEASON Urinary cadmium concentration $\frac{\mu mol\ Cd}{mol\ creat}$	END-SEASON Number of subjects	END-SEASON Urinary cadmium concentration ng/ml	END-SEASON Urinary cadmium concentration $\frac{\mu mol\ Cd}{mol\ creat}$
I	14	2.04 ±0.81	2.18 ±2.02	11	1.92 ±1.12	1.90 ±1.84
II	21	1.71 ±1.03	1.57 ±1.21	19	1.99 ±1.13	1.77 ±1.29
III	21	2.55 ±1.78	2.03 ±1.87	20	2.90 ±2.17	2.37 ±1.98
IV	7	1.98 ±1.64	1.44 ±0.54	7	1.74 ±0.97	1.67 ±0.83

Values are mean ± SD

Smoking did not influence consistently the cadmium concentration in urine as can be seen from the end-season data which is summarised in Table 42. For Category I and Category II subjects the non-smokers had a higher (about 1 ng/ml) cadmium concentration than did the smokers, while the reverse was true for Category III and Category IV subjects, indicating that smoking probably did not have a major influence on urinary cadmium concentration.

For those subjects who participated at both sampling times the urinary cadmium data is summarised in Table 43. As for the data for all subjects (Table 41) there was no consistent trend in urinary cadmium concentration among the categories with an increasing oyster consumption, either pre-season or end-season. Further, excluding Category IV subjects for which only 3 of them produced a urine sample at both times, there was an apparent increase in cadmium concentration within each category at the end of the season, but this was not statistically significant because of the great individual variations.

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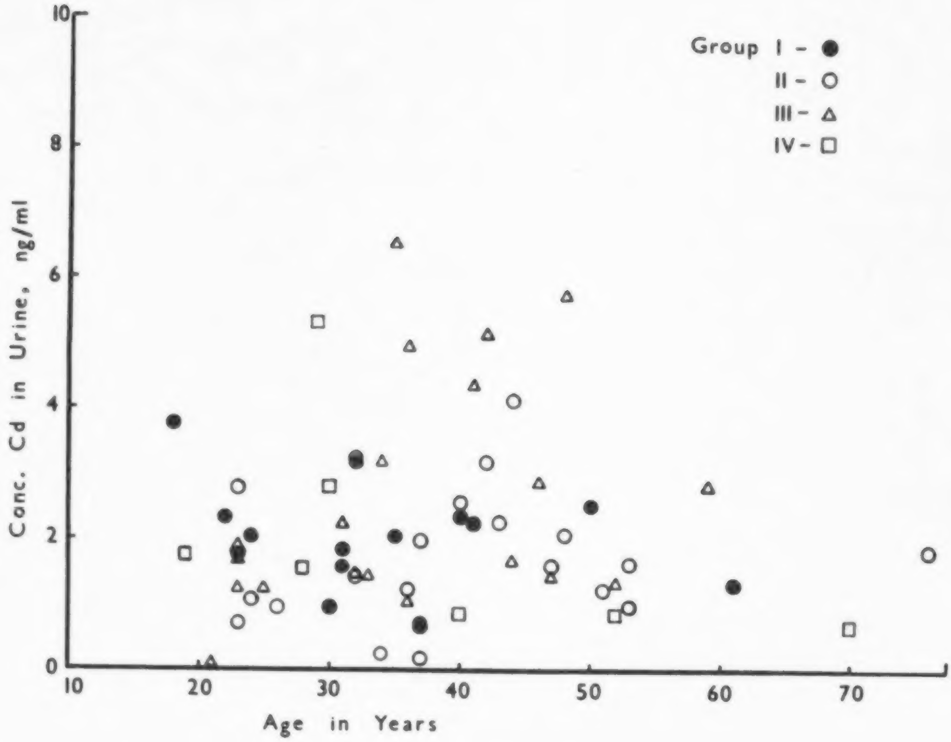

FIGURE 17: END-SEASON URINARY CADMIUM CONCENTRATION COMPARED WITH THE AGE OF THE SUBJECTS BY OYSTER INTAKE CATEGORY: ● = CATEGORY I: O = CATEGORY II; △ = CATEGORY III, □ = CATEGORY IV.

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TABLE 42. SUMMARY OF END-SEASON URINARY CADMIUM CONCENTRATION FOR NON-SMOKERS AND FOR SMOKERS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	URINARY CADMIUM CONCENTRATION, ng/ml* NON-SMOKERS Number of subjects	x̄ ± SD	SMOKERS Number of subjects	x̄ ± SD
I	6	2.17±1.28	5	1.63±0.94
II	14	2.09±1.01	5	1.71±1.51
III	10	2.27±1.73	10	3.53±2.47
IV	2	0.85(±0.60)	5	2.10±0.87

TABLE 43. SUMMARY OF URINARY CADMIUM CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	URINARY CADMIUM CONCENTRATION, µmol Cd/mol creatinine PRE-SEASON Number of subjects	x̄ ± SD	END-SEASON Number of subjects	x̄ ± SD
I	5	1.54±0.66	5	1.68±1.54
II	17	1.35±0.37	12	1.78±1.37
III	16	1.97±0.31	16	2.33±2.09
IV	3	1.67(±0.31)	3	1.25(±0.61)

6.4.3. Urinary Zinc Excretion

Appendices K1-K4 present the individual data, which are summarised in Table 44 for all subjects; urinary zinc concentration has been expressed both as an uncorrected concentration (µg/ml) and corrected to the creatinine content (mmol Zn/mol creatinine). There was no consistent trend among the categories in the pre-season concentration of zinc, nor was there a trend at the end of the season, except that Category IV subjects had a greater variability and a slightly higher mean concentration expressed as mmol Zn/mol creatinine. Within each category of oyster intake there was no difference

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in the zinc concentration at either sampling time, except again that Category IV subjects might have had a slightly higher corrected zinc concentration. However since urinary zinc excretion represents a minor proportion of the dietary intake, and since urinary zinc excretion does not usually reflect variations in dietary zinc intake, a greater number of subjects would be needed to confirm whether a large consumption of oysters could raise urinary zinc excretion. It was noted that Category IV subjects were all men, and that they had a greater body weight than did the other categories (Table 18), and these two factors could have contributed to a slightly greater urinary zinc excretion, as has been reported previously for New Zealand men (McKenzie, 1979).

TABLE 44. SUMMARY OF URINARY ZINC CONCENTRATION BY OYSTER INTAKE CATEGORY*

OYSTER INTAKE CATEGORY	PRE-SEASON			END-SEASON		
	Number of subjects	Urinary zinc concentration		Number of subjects	Urinary zinc concentration	
		μg/mol	mmol Zn / mol creat		μg/ml	mmol Zn / mol creat
I	13	0.57 ±0.41	0.73 ±0.29	11	0.54 ±0.24	0.67 ±0.31
II	21	0.44 ±0.27	0.61 ±0.28	19	0.56 ±0.33	0.69 ±0.32
III	21	0.62 ±0.33	0.77 ±0.33	20	0.51 ±0.25	0.64 ±0.29
IV	7	0.65 ±0.47	0.67 ±0.30	7	0.57 ±0.37	1.07 ±0.58

Values are mean ± SD

The urinary data for those subjects who participated at both sampling times is summarised in Table 45. Again there was no consistent change in urinary zinc concentration either among categories pre-season or end-season, or within categories pre-season and end-season, except that again the three subjects in Category IV appeared to have a higher zinc concentration at the end of the season. The same comments apply to these three subjects as were made about all the subjects in Category IV (Table 44) at the end of the season.

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TABLE 45. SUMMARY OF URINARY ZINC CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	URINARY ZINC CONCENTRATION, mmol Zn/mol creatinine* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
I	8	0.81±0.30	8	0.73±0.32
II	17	0.56±0.26	17	0.70±0.33
III	18	0.75±0.34	18	0.65±0.31
IV	3	0.57±0.19	3	1.08±0.71

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6.4.4. Urinary Beta-2-microglobulin Concentration

Appendices K1-K4 present the indivudual data, which has been corrected to a specific gravity of 1.025. All values were within the normal range for healthy adults of 4-370 μg/l (Shiroishi et al., 1977). As can be seen from the summary in Table 46 there was no difference among categories pre-season or end-season except that Category IV subjects had a slight higher pre-season mean. The higher mean value of 106 μg/l was because of the high concentration for one subject (31.4.AF: 254 μg/l); his end-season concentration was lower at 115 μg/l. Category IV subjects at the end of the season did not have a mean beta-2-microglobulin concentration different from the other categories.

Within each category there was no consistent change from the beginning to the end of the season in their beta-2-microglobulin concentration.

For the smaller number of subjects who gave a urine sample at both times (Table 47) again the only higher value was the mean of 162 μg/l for Category IV subjects pre-season because of the higher concentration in the urine of subject 31.4.AF.

From these data it can be seen that urinary beta-2-microglobulin concentration was normal in all subjects and was unaffected by the consumption of oysters.

6.5. Hair Data

Appendices L1-L4 present the data for the concentration of cadmium, zinc and copper in the hair of all subjects.

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TABLE 46. SUMMARY OF URINARY BETA-2-MICROGLOBULIN CONCENTRATION FOR SUBJECTS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	URINARY BETA-2-MICROGLOBULIN* CONCENTRATION, μg/l PRE-SEASON Number of subjects	$\bar{x} \pm SD$	END-SEASON Number of subjects	$\bar{x} \pm SD$
I	15	64±41	11	75±42
II	18	84±70	20	72±42
III	21	80±40	20	57±29
IV	9	106±67	5	58±43

Values have been corrected to specific gravity 1.025

TABLE 47. SUMMARY OF URINARY BETA-2-MICROGLOBULIN CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	URINARY BETA-2-MICROGLOBULIN* CONCENTRATION, μg/l PRE-SEASON Number of subjects	$\bar{x} \pm SD$	END-SEASON Number of subjects	$\bar{x} \pm SD$
I	8	77±46	8	66±44
II	16	82±74	16	76±42
III	18	80±43	18	55±26
IV	3	162±87	3	83±35

6.5.1. Hair Cadmium Concentration

The mean hair cadmium concentration for all subjects at the beginning of the season was 0.42±0.24 μg/g (n=53) and 0.24±0.18 μg/g at the end of the season; men (n=39) had a higher end-season hair cadmium concentration (0.27±0.19 μg/g) than did women (n=11) (0.11±0.07). However it has been

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reported (Whanger, 1979) that smokers have a higher hair cadmium concentration (1.66±0.45 μg/g) than do non-smokers (0.99±0.38 μg/g) but this was not found to be true for the present study. The non-smokers (n=23) had a mean hair cadmium concentration of 0.24±0.14 μg/g while the smokers (n=25) had a mean of 0.25±0.21 μg/g). Since there was no difference the smokers and non-smokers were combined in the calculation of the mean values for each oyster intake category (Table 48). There was no difference among the means for the pre-season values, and a trend towards higher values for Categories III and IV at the end of the season. When the end-season mean was compared to the pre-season mean within each category there was an apparent decrease for Categories I, II and III, but no change for Category IV.

TABLE 48. SUMMARY OF MEAN HAIR CADMIUM CONCENTRATION BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	HAIR CADMIUM CONCENTRATION, μg/g* PRE-SEASON Number of subjects	$\bar{x} \pm$ SD	END-SEASON Number of subjects	$\bar{x} \pm$ SD
I	13	0.35±0.22	10	0.18±0.11
II	17	0.44±0.25	16	0.18±0.13
III	17	0.47±0.27	14	0.29±0.17
IV	6	0.37±0.22	8	0.36±0.28

Again there was no difference among the categories for the pre-season hair samples for those subjects who gave hair at both sampling times (Table 49); neither was there a difference among the means for the end-season samples, except that Category III subjects had a slightly higher mean. Comparison of the end-season means with the pre-season means within each category again showed a decreased mean at the end of the season for all categories.

On first consideration it was not obvious why the end-season values should have been lower, especially since the blood cadmium concentration had increased at the end of the season for most subjects, although not for all of the smokers (Table 32). The decreased concentration at the end of the season held true for both the smokers and the non-smokers. Consideration of the hair sampling procedure and of the physiology of hair growth might provide a possible explanation. About 5 mm hair would have been left next to the scalp when the hair was cut with the scissors. Hair grows at about 10 mm per month and it would take about another month for hair to be extruded from the hair follicle through the surface of the scalp. Therefore there

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would be about one and a half month's time lag before the hair representing the first day of the oyster season would be in the segment of hair being cut. It would take another two months before the whole segment of hair cut represented hair grown since the beginning of the oyster season (average length of hair analysed was 20 mm). After a sudden increase in cadmium intake, it takes two to three months before the peak concentration is reached in blood (Kjellstrom & Nordberg, 1978). Therefore it could take 5-7 months after a change in cadmium intake till the hair being analysed achieved its minimum or maximum concentration. Thus perhaps the hair samples taken in February (pre-season) better represented the end of the oyster season (August) in the year before, and the hair samples taken early in August better represented the beginning of the oyster season when the blood concentrations were still low.

TABLE 49. SUMMARY OF HAIR CADMIUM CONCENTRATION FOR SUBJECTS WHO GAVE A HAIR SAMPLE BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	HAIR CADMIUM CONCENTRATION, µg/g *			
	PRE-SEASON		END-SEASON	
	Number of subjects	$\bar{x} \pm$ SD	Number of subjects	$\bar{x} \pm$ SD
I	8	0.40±0.26	8	0.18±0.11
I	12	0.45±0.22	12	0.18±0.15
III	12	0.49±0.27	12	0.27±0.18
IV	4	0.47±0.19	4	0.22±0.12

In any case, it was only a two-fold difference in the mean values (Table 49) and all categories of oyster intake were affected. Analysis of serial hair samples taken over a whole year from people with a high seasonal oyster intake might clarify this finding. These results point to some of the difficulties in using hair analysis for the study of cadmium exposure.

6.5.2. Hair Zinc Concentration

Appendices L1-L4 present the individual hair zinc data. All subjects were questioned about their use of anti-dandruff shampoos since some contain zinc. The mean hair zinc concentration for all subjects pre-season (n=61) was 195±43 µg/g and end-season (n=52) was 169±23 µg/g. Men (n=41) had a similar hair zinc concentration, 171±24 µg/g, to that for the women (n=11), 162±17 µg/g (end-season values). Smoking did not influence hair zinc concentration.

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The hair zinc concentrations for all subjects are summarised by oyster intake category in Table 50. There was no difference in the mean value among the categories for either the pre-season or the end-season values, but comparison within each category indicated a slightly decreased value at the end of the season.

TABLE 50. SUMMARY OF MEAN HAIR ZINC CONCENTRATION BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	HAIR ZINC CONCENTRATION, μg/g* PRE-SEASON Number of subjects	PRE-SEASON $\bar{x} \pm$ SD	END-SEASON Number of subjects	END-SEASON $\bar{x} \pm$ SD
I	15	195±41	11	169±19
II	19	195±34	18	173±28
III	20	189±39	15	173±18
IV	7	208±75	8	155±20

TABLE 51. SUMMARY OF HAIR ZINC CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	HAIR ZINC CONCENTRATION, μg/g* PRE-SEASON Number of subjects	PRE-SEASON $\bar{x} \pm$ SD	END-SEASON Number of subjects	END-SEASON $\bar{x} \pm$ SD
I	8	184±42	8	162±14
II	14	191±30	14	168±23
III	13	189±37	13	173±19
IV	4	199±49	4	159±16

The slightly decreased concentrations at the end of the season were also observed for those subjects who gave hair samples at both times (Table 51); however the difference was not more than 40 μg/g. Although the same explanation might apply to the zinc values as for the decreased hair cadmium values, this seemed less likely since serum zinc concentration had not increased at the end of the season and since all categories of oyster intake were affected. Furthermore, dosing with $ZnSO_4$ for 30 days did not appear to have increased hair zinc concentration in a previous experiment (McKenzie, 1978).

6.5.3. Hair Copper Concentration

Appendices L1-L4 present the individual data. The average pre-season hair copper concentration for all subjects (n=60) was 17.8 ± 9.5 µg/g, and at the end of the season (n=52) was 18.2 ± 9.2 µg/g. Men (n=41) had a mean value of 16.8 ± 6.5 µg/g while women (n=10) had a mean hair copper of 23.4 ± 15.0 µg/g; exclusion of the subject on an oral contraceptive reduced the mean value for women (n=10) to 21.3 ± 14.1 µg/g. Table 52 summarises the data for all subjects by oyster intake category, and shows that there was no consistent difference within categories between the beginning and the end of the season. Table 53 confirms that this lack of differences held true for those subjects who gave hair samples at both times; although Category IV subjects appear to have a lower hair copper, the number of subjects (3) in the category was too few to draw any conclusions.

TABLE 52. SUMMARY OF HAIR COPPER CONCENTRATION BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	HAIR COPPER CONCENTRATION, µg/g*			
	PRE-SEASON		END-SEASON	
	Number of subjects	$\bar{x}$ ± SD	Number of subjects	$\bar{x}$ ± SD
I	15	17.7 ± 9.7	11	20.1 ± 8.5
II	19	18.8 ± 10.4	18	15.0 ± 7.0
III	20	19.1 ± 9.6	15	20.5 ± 12.1
IV	6	12.4 ± 3.4	8	16.4 ± 8.4

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TABLE 53. SUMMARY OF HAIR COPPER CONCENTRATION FOR SUBJECTS WHO GAVE SAMPLES BOTH PRE-SEASON AND END-SEASON

OYSTER INTAKE CATEGORY	HAIR COPPER CONCENTRATION, μg/g* PRE-SEASON Number of subjects	$\bar{x}$ ± SD	END-SEASON Number of subjects	$\bar{x}$ ± SD
I	8	16.2± 7.5	8	17.9± 5.9
II	14	18.8±11.8	14	17.1± 7.2
III	13	18.6± 8.6	13	20.0±12.7
IV	3	10.9± 4.0	3	13.2± 5.9

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6.6. Medical History and Blood Pressure Data

6.6.1. Medical Histories

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The subjects in this study were generally healthy. The only chronic diseases admitted to were arthritis (3.1.HA); rheumatism (13.3.RB; 43.1.PK); chronic bronchitis (13.3.RB; 20.3.CC); asthma (16.1.MB; 6.2.WB; 28.3.MD); diabetes (45.1.RK); hypertension (45.1.RK; 13.3.RB).

6.6.2. Blood Pressure Data

The individual systolic blood pressure (SBP) and diastolic blood pressure (DBP) observed for subjects is presented in Appendix F, and the mean values by oyster intake category are summarized in Table 54. Both SBP and DBP were higher for Category IV subjects than for the other subjects; however the body weight and body mass index (Quetelet's index) was greater for Category IV subjects (Table 18) who also smoked more cigarettes.

Table 54 also contains the mean expected blood pressures for each category. The expected values were derived from the relationship between age, body mass index (BMI) and SBP and DBP for men and for women from the health survey carried out in Milton in 1981. (Milton is a rural township 100 miles north of Bluff, and is considered to be statistically representative of a New Zealand semi-rural population). The equations were:

Men (n = 548)
SBP = 0.4368 age + 0.6711 BMI + 94.6728
DBP = 0.1723 age + 0.8559 BMI + 51.7970

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Women (n = 589):

$$SBP = 0.8196\ age + 0.2038\ BMI + 82.2134$$
$$DBP = 0.3010\ age + 0.2064\ BMI + 58.5421$$

where SBP, DBP are 3rd readings
DBP is Phase IV
BMI is body mass index (kg/m^2) = weight/$(height)^2$

The calculated blood pressures showed the expected increase in systolic and in diastolic blood pressure because of the greater body mass of Category III and especially Category IV subjects. The observed blood pressures were greater than were expected from the age and body size of Category III and Category IV subjects, indicating that some other factor(s) might have been contributing to their observed pressures. Two subjects were under treatment for hypertension (13.3 RB and 45.1 RK, Appendix F) but their blood pressures were still higher than the group means.

TABLE 54. SYSTOLIC BLOOD PRESSURE AND DIASTOLIC BLOOD PRESSURE FOR SUBJECTS BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	NUMBER OF SUBJECTS	BLOOD PRESSURE, mm Hg			
		OBSERVED		PREDICTED	
		SBP	DBP	SBP	DBP
I	12	131±23	81±15	125±9	79±5
II	18	129±16	82±12	129±6	80±4
III	20	137±14	84±15	128±6	82±4
IV	8	142±22	88±13	132±7	84±4

In order to adjust the individual blood pressure measurements for age and body mass a "blood pressure ratio" (BPR) was calculated. This is the ratio between observed and expected blood pressure as calculated above. A BPR of 1.00 indicates that this individual has a blood pressure identical to the average for his/her age and body mass group in Milton. Using the BPR variable the relationship between systolic blood pressure and end-season blood cadmium was analysed for smokers and non-smokers. In the smoking group (n=32) the correlation coefficient between the two variables was + 0.33 (p = 0.037, 1-tailed) within the blood cadmium range 1-12 μg/l. For the non-smoking group (n=26) the correlation coefficient was -0.04 within the blood cadmium range 0.9 - 4 μg/l.

The average BPR was similar, 1.07 for smokers and 1.05 for non-smokers. As shown in Section 6.3.2. the blood cadmium level increased with oyster intake for each of the two groups, but the increase due to oyster intake alone (up to 4 μg/l) was not associated with a blood pressure increase. Whether the apparent increase of blood pressure at blood cadmium levels in the range 4 - 12 μg/l is among smokers is due to cadmium or some other

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factor in smoke can not be evaluated from these data. It is interesting to note that among smokers with blood cadmium below 4 μg/l the average BPR (0.99) tended to be lower than for non-smokers (1.05) and lower than for the other smokers (1.10).

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SECTION 7

DISCUSSION OF INDICATORS OF CADMIUM INTAKE AND DOSE

7.1. Relationship Between Cadmium Intake From Oysters and Faecal Cadmium Output

Almost all cadmium ingested orally is excreted in the faeces. Only a minor proportion, approximately 5%, appears to be absorbed (Nordberg, 1976; Shaikh & Smith, 1980), and thus the faecal output of cadmium will reflect the oral intake of cadmium. Some absorbed cadmium probably is re-excreted into the gastro-intestinal tract in man; however the amount would be small compared to the total amount (exogenous and endogenous) of cadmium in the faeces.

The preliminary study (Section 6.2.1.) indicated that after a single high intake of cadmium from oysters faecal excretion had returned to baseline values within 5 days i.e. there was not any unusual handling of cadmium in the gut contents. This excretion of unabsorbed cadmium from the gut within a few days of ingestion was in close agreement with earlier reports on orally ingested cadmium in human subjects (McLellan et al., 1978; Shaikh and Smith, 19809).

For each category of oyster consumers the mean end-season consumption of oysters (Table 16) was closely related ($r = 0.97$) to the mean daily faecal output of cadmium (Table 22) as is shown in Figure 18. The intercept at a zero intake of oysters was 14.5 µg Cd which agreed very closely with the estimated daily intake of 15 µg cadmium (Table 4) in a normal New Zealand diet. Assuming an intake of 15 µg cadmium per day from normal foods, as well as the additional intake of cadmium from oysters using the end-season consumption data, the calculated intake of cadmium in comparison with the observed output for each category is presented in Table 55. There was close agreement between the calculated intake and the observed faecal output for each oyster intake category, confirming that the subjects were ingesting the numbers of oysters they reported, and that if, as for other foods, only a minor amount of absorbed cadmium is re-excreted into the gut, then only a small proportion of the intake apparently was absorbed. True absorption cannot be calculated from the data in this study.

The mean daily faecal excretions of cadmium in Categories I and II were similar to those reported in the studies of Tipton et al., 1969 (30 and 47 µg/d), Tsuchiya, 1969 (57 µg/d) and Tati et al., 1976 (41.1-79.4 µg/d) for people not exposed to high amounts of cadmium.

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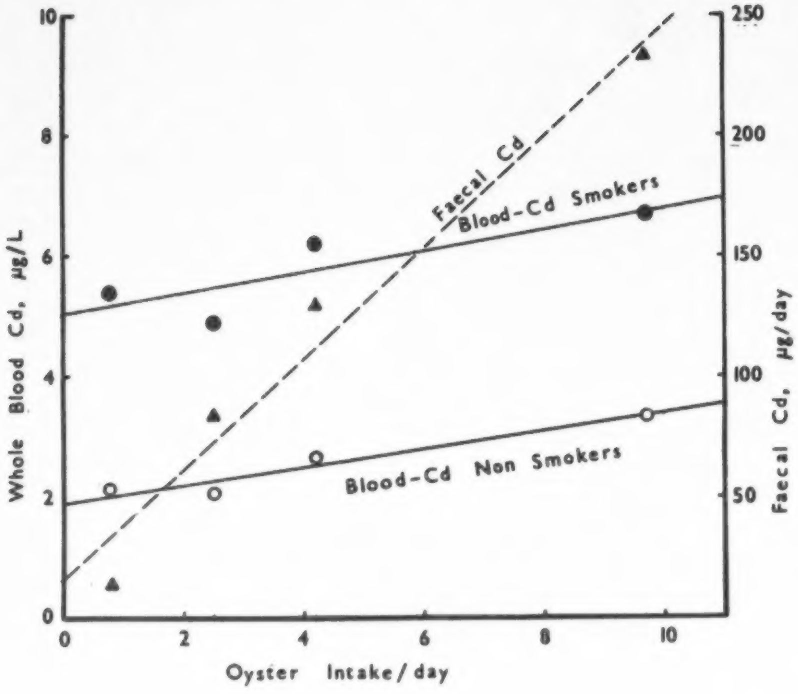

FIGURE 18: RELATIONSHIP BETWEEN MEAN END-SEASON CONSUMPTION OF OYSTERS AND MEAN FAECAL CADMIUM OUTPUT (▲), MEAN WHOLE BLOOD CADMIUM FOR NON-SMOKERS (O) AND MEAN WHOLE BLOOD CADMIUM FOR SMOKERS (●), BY OYSTER INTAKE CATEGORY.

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TABLE 55. COMPARISON OF CALCULATED TOTAL MEAN END-SEASON CADMIUM INTAKE WITH THE OBSERVED MEAN END-SEASON FAECAL CADMIUM OUTPUT BY OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	CALCULATED TOTAL INTAKE µg Cd	OBSERVED DAILY FAECAL CADMIUM OUTPUT µg Cd
I	34	15
II	75	84
III	116	129
IV	248	233

Although there would inevitably have been errors in the collection of the 3-day faecal samples, on a group basis (i.e. by category) it was considered reasonable to calculate a mean daily output from those 3-day collections. The data confirmed the use of faecal output of cadmium as an indicator of oral cadmium intake.

7.2. Relationship Between Cadmium Intake from Oysters and Blood Cadmium Concentration

Since smoking affected whole blood cadmium concentration, the relationship between mean end-season oyster intake and mean end-season whole blood cadmium concentration has been plotted separately for smokers and for non-smokers in Figure 18. The correlation between mean oyster intake and mean blood cadmium was good for both the non-smokers ($r = 0.97$) and the smokers ($r = 0.84$). Extrapolation of the correlation for the non-smokers gave an intercept of 1.9 ng/ml at a zero oyster intake. This was about twice as high as the mean for the control group (0.90 ng/ml, Table 29). Two factors might have contributed to this higher mean whole blood cadmium concentration in the non-smokers:

(a) passive smoking - the oystermen regularly socialised together in an environment with a high density of cigarette smoke and minimal ventilation.

(b) ex-smokers - some of the subjects classified as non-smokers were ex-smokers and their blood cadmium concentration might therefore have been higher than for true non-smokers, e.g. 27.3.BD had given up smoking 3 weeks prior to taking his blood sample.

The effect of smoking on whole blood cadmium was consistent for all categories, and was greater than the effect of eating 10 oysters per day. On average smoking resulted in a 2-3 times increase in blood cadmium, which is a greater effect than has been reported previously (Kjellstrom, 1979);

indeed no effect of smoking on blood cadmium was reported in a study of some Oregon residents (Whanger, 1979). New Zealand cigarettes appear to have a total cadmium content similar to overseas brands (Brooks & Trow, 1979); but it might be that the efficiency of the filters in New Zealand cigarettes is not as good.

The total calculated intake of cadmium at the end of the season for Category IV subjects (Table 55) was 16 times the normal daily intake of 15 μg, and was 8 times the normal daily intake for Category III subjects. However, the enhancement in blood cadmium concentration was barely a 50% increase for both the non-smokers and the smokers in Category IV. This effect was less than expected in the light of the large increase in dietary intake.

7.3. Relationship Between Cadmium Intake from Oysters and Urinary Cadmium Concentration

Urine is thought to be the major excretory route for absorbed cadmium although the proportion of the body burden excreted daily in the urine is very small (0.0075% per day, Elinder et al., 1978). Both animal and human studies have indicated that urinary excretion of cadmium might increase as tissue accumulation (i.e. renal accumulation) increases in the phase prior to detectable renal damage (Norberg, 1976). Following cadmium-induced renal tubular impairment the urinary excretion of cadmium increases markedly. Thus the urinary excretion of cadmium could be used to indicate whether a subject had been exposed to higher than usual levels of cadmium.

Table 56 summarises the mean calculated intake of cadmium, and the observed urinary concentration of cadmium for each oyster intake category (Table 41). Even despite a 16-fold increase in the usual dietary intake of cadmium, Category IV subjects did not have an increased urinary cadmium concentration. Furthermore there was no relationship between age and urinary

TABLE 56. SUMMARY OF CALCULATED TOTAL END-SEASON MEAN CADMIUM INTAKE COMPARED WITH THE OBSERVED END-SEASON MEAN URINARY CADMIUM CONCENTRATION

OYSTER INTAKE CATEGORY	CALCULATED TOTAL INTAKE μg/d	URINARY CADMIUM CONCENTRATION	
		ng/ml	μmol/mol creatinine
I	34	1.92	1.90
II	75	1.99	1.77
III	116	2.90	2.37
IV	248	1.74	1.67

cadmium concentration (Figure 17); indeed the 70-year old Category IV subject (65.4.TS) had a urinary concentration of 0.43 ng/ml (1.32 µmol/mol creatinine) at the end of the season, i.e. a concentration at the lower end of the concentrations for all the subjects. Data obtained in a Japanese study of subjects without a known exposure to cadmium in industry or from cadmium-contaminated foods or beverages indicated an increased urinary cadmium concentration with increasing age (Tati et al., 1979).

Therefore on the basis of urinary cadmium concentrations in the present study it seemed that the ingestion of cadmium from the oysters had not resulted in the accumulation of sufficient cadmium in their renal tissue to result in an increased urinary cadmium excretion.

7.4. Relationship Between Cadmium Intake from Oysters and Hair Cadmium Concentration

The hair cadmium data was difficult to interpret because hair cadmium concentration appeared to have decreased at the end of the season, although there was no difference among the categories either pre-season or at the end of the season (Table 48). Thus there did not seem to be any immediately discernible relationship between cadmium intake and hair cadmium concentration in this study. The seasonal character of the high cadmium intake via oysters would involve a similar seasonality in the hair cadmium levels. Because of the "delay" in hair growth and cadmium accumulation in blood (see section 6.5.1), a lower hair cadmium level at the end of the season would still be compatible with a correlation between long-term cadmium intake and hair level. However, the lack of a correlation between oyster intake and hair cadmium level speaks against hair being used as an indicator of cadmium intake.

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SECTION 8

DISCUSSION OF EFFECTS OF CADMIUM

The effects of excessive exposure to cadmium from food sources have been studied in Japan. Proteinuria and glycosuria were the most common effects (Ishizaki, 1969; Shiroishi et al., 1972) i.e. renal damage. Evidence from several animal studies (both rats and rabbits) has indicated that the chronic ingestion of small doses of cadmium can induce hypertension (Schroeder, 1967; Thind, 1972) however there is no direct evidence that this would hold true for man.

8.1. Renal Effects of Cadmium Ingestion From Oysters

It is generally considered that renal tubular damage is the critical effect of cadmium in human subjects after long-exposure (WHO, 1977). Epidemiological studies have indicated that at an average long-term intake of about 200 µg cadmium per day a response rate of 5-20% renal tubular damage can be expected (Kjellstrom, 1977). Urinary B_2-microglobulin excretion was used as the sensitive indicator of such damage.

In the present study all subjects had a urinary B_2-microglobulin concentration within the normal range, and there was no consistent change with cadmium intake (Table 46). Figure 19 also illustrates this lack of a relationship between urinary cadmium concentration, urinary B_2-microglobulin concentration, or serum B_2-microglobulin concentration with oyster intake, using the mean end-season oyster intake data.

Thus there was no indication of any renal damage from the parameters measured in the present study. This seemed to be in disagreement with the predicted rate of cadmium - induced renal impairment, since Category IV subjects had a calculated mean intake of 248 µg cadmium per day at the end of the season. The seasonal average intake of oysters was even higher than the average end-season intake, but perhaps the seasonal nature of the exposure (6 months' ingestion followed by 6 months' abstention) had an ameliorating effect in terms of the resulting damage. Other possible explanations are discussed in Section 9 i.e. interactions with other trace elements.

The analysis of tissue obtained at autopsy from Bluff residents including from those who had eaten high numbers of oysters during their lifetime would help to determine the degree of accumulation of cadmium in the tissues, especially in renal tissues.

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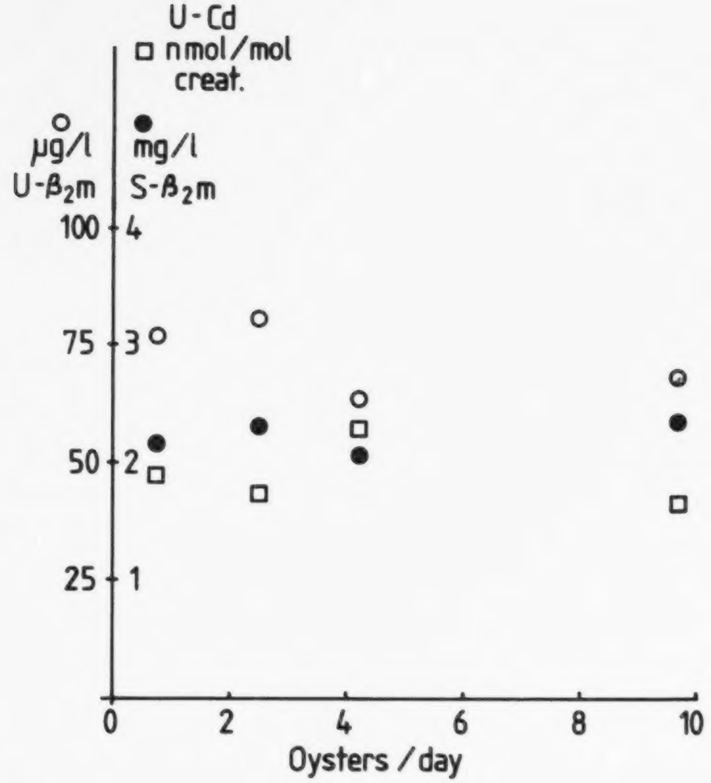

FIGURE 19: RELATIONSHIP BETWEEN MEAN URINARY CADMIUM CONCENTRATION (μmol/mol creatinine; ☐), MEAN URINARY B_2-MICROGLOBULIN CONCENTRATION (μg/l; 0) AND MEAN SERUM B_2-MICROGLOBULIN CONCENTRATION (mg/l; ●), AND MEAN END-SEASON OYSTER INTAKE FOR THE FOUR CATEGORIES OF OYSTER INTAKE.

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The World Health Organisation (1972) proposed a provisional tolerable weekly intake of 400-500 µg cadmium per individual, based on their available data on the metabolism of cadmium (i.e. intake, absorption, retention, excretion). The mean weekly intake of cadmium for each oyster intake category based on their end-season oyster intake data and a normal intake of 15 µg/d is presented in Table 57. An intake of 15 µg cadmium per day is equivalent to a weekly intake of 105 µg; as can be seen from Table 57 only this normal intake and the intake of Category I subjects fall below the WHO provisional tolerable intake. While it was comforting from a public health point of view to find that the data in the present study consistently indicated no renal efects from these high intakes, it is important to further investigate whether any tissue accumulation, and especially any renal accumulation, is occurring in these subjects. Such an investigation would greatly advance our present knowledge of the metabolism of cadmium in man.

TABLE 57. CALCULATED TOTAL MEAN END-SEASON DAILY AND WEEKLY INTAKE OF CADMIUM FOR EACH OYSTER INTAKE CATEGORY

OYSTER INTAKE CATEGORY	CALCULATED MEAN CADMIUM INTAKE µg/d	µg/week
I	34	238
II	75	525
III	116	812
IV	248	1736

8.2. Effect of Cadmium Ingestion From Oysters on Blood Pressure

It has been shown that under certain environmental and nutritional exposure conditions, cadmium will induce hypertension in animals (Perry et al., 1977; Whanger, 1979). For humans only a limited number of studies have been carried out to examine the relationship between cadmium exposure and hypertension. While some studies (Frigerg, 1950; Holden, 1980) of cadmium exposed workers show no indications of an increased incidence of hypertension some studies suggest that cadmium may be a causative factor in the development of these diseases (Schroeder, 1965; Vorobjeva & Eremeeva, 1980). Other recent studies (Ostergaard, 1978; Cummins et al., 1980) indicate that cadmium in itself may not necessarily be a causative factor in the development of cardiovascular disease and other factors such as cigarette smoking may be an important factor.

There are very few results available from studies on chronic cadmium ingestion and effects on blood pressure in the general population.

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Available data on the cardiovascular effects of cadmium are not conclusive (Nogawa & Kawano, 1969).

In the present study the observed blood pressures were higher than those predicted (due to age and body mass) in the highest oyster intake categories (Table 54). An analysis of the relationship between blood pressure and blood cadmium (Section 6.6.2.) for smokers and non-smokers separately showed that at blood cadmium levels above 4 μg/l there may be an increased blood pressure associated with blood cadmium. As such high blood cadmiums only occurred among smokers, it is uncertain whether cadmium itself is a causative factor.

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SECTION 9

DISCUSSION OF INTERACTIONS BETWEEN CADMIUM AND OTHER TRACE ELEMENTS

Much remains to be learned of the interactions of cadmium with other trace elements, but it is widely recognised that due regard must be given to the dietary intake of several essential trace elements when the toxicity of cadmium is being considered. In the present study zinc, copper and selenium were the main trace elements investigated in addition to cadmium; the effects of the iron status and the calcium status will also be considered briefly. Very little can be deduced about interactions that might have influenced the effects of the intake of cadmium from oysters from the data obtained in this study.

9.1. Cadmium, Zinc and Copper Interactions

Cadmium, zinc and copper are chemically related in that they belong to Group IIB in the Periodic Table. An interaction between these three elements would therefore be predicted according to Hill and Matrone's hypothesis (1970) which states that elements which have similar chemical and physical properties will interact antagonistically with each other biologically. Experimental data with rats confirmed an antagonistic interaction between these three elements, the strongest antagonism being between zinc and copper.

It is well established that an increased level of intracellular zinc prior to exposure to cadmium plays an important role in the detoxification of the toxic metal, particularly in the liver, kidney and testicular tissues. Although the precise mechanisms of the cadmium-zinc interactions in human tissues are not known, animal experimental data indicate that the interactions may be through an induction of metallothionein (MT) synthesis and its subsequent binding to zinc and cadmium. Similarly, cadmium treatment may cause an accumulation of MT-bound copper in the tissues particularly in the kidney. In this context, the additional intake of copper via Bluff Oysters may enhance the secondary accumulation of copper in the kidney tissues.

In the present study, if the high intake of cadmium were causing an accumulation of zinc and/or copper in liver and in kidney, it is not certain whether this accumulation should have resulted in higher serum concentrations or in a greater urinary excretion. Serum concentration is not a good indicator of status either for zinc or for copper, nor is urinary excretion thought to be a good indicator of zinc status except at high dietary intakes (McKenzie, 1979). Neither serum zinc nor serum copper concentration differed among the four categories of oyster intake, but it was noted that there was

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a reverse trend in the serum zinc and copper values when the end-season means were compared with the pre-season means. Whether this trend was indicative of an interaction between cadmium and/or zinc or copper, or between zinc and copper cannot be determined. Only for Category IV at the end of the season did urinary zinc excretion appear to increase, but since there were only 3 subjects in Category IV who participated in both the pre-season (0.57 µmol Zn/mol creatinine) and the end-season sampling (1.08 µmol Zn/mol creatinine) a valid comparison was not possible.

If cadmium and zinc had been interacting at the site of absorption such as to inhibit one another, then the expected and the observed faecal outputs both of cadmium and of zinc might not have agreed as remarkedly as they did (Figure 12). Since the faecal outputs were so well predicted by recent oyster intake an interaction between the two elements in the gut (i.e. during absorption or during endogenous excretion) seemed less likely.

Since faecal output of copper did not increase with an increasing oyster consumption, and since the expected increase was only slight, an interaction between cadmium and copper also appeared unlikely. Some studies have indicated that a marked species difference might exist in response to cadmium and zinc ingestion, at least between the lamb and the rat. In lambs zinc tends to alleviate the toxicity of cadmium, but in rats the severity of the copper deficiency induced by cadmium was increased by zinc supplementation (Bremer, 1974).

Oysters enhanced the dietary trace element intake by different proportionate amounts (Table 4), with the cadmium intake being enhanced to a much greater extent than for zinc, copper or manganese. Recent studies have placed a greater emphasis on low levels of supplementation, i.e. levels similar to those more likely to be encountered in human diets. Under certain dietary and physiological conditions even relatively low doses can markedly disturb zinc, copper and iron metabolism, and even when these diets are well-fortified with these essential metals. Similar studies with human subjects would be very difficult to carry out, and while there have been a few reports of ratios and correlations among metals in various samples and tissues, it is not known whether chronic low-level exposure to cadmium would lead to direct interactions with other metals in human fluids and tissues. An investigation of autopsy tissues in the Bluff population surveyed in the present study would help to determine likely interactive effects caused by chronic cadmium ingestion from oysters. Also, metabolic studies using oysters with different proportions of trace elements (such as are found among different species in New Zealand, Table 3) would help to elucidate the question of interactions.

9.2. Cadmium - Selenium Interactions

The most efficient known protector against cadmium-induced testicular damage is selenium, which has been shown to divert the binding of cadmium from low molecular weight proteins to higher molecular weight proteins in the testes of rats. As well, nearly all of the cadmium binding in plasma was diverted by selenium from low molecular weight proteins to high molecular weight proteins; about 50% of the cadmium binding in kidneys and only a small

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amount of cadmium binding in the liver was diverted by selenium.

The chemical form of selenium appeared to be important in diverting the cadmium binding in tissue, in that selenate and selenite were more effective than selenomethionine, which had little or no effect. However, the amount of cadmium accumulated in the testes was greater in the presence than in the absence of selenium, even though the toxic effect was reduced (Whanger et al., 1981).

Caution must be exercised in extrapolating the results of the above studies, because the effects were produced after injection of selenium; neither oral dosing nor inclusion of selenium or cadmium in the diet resulted in altered cadmium binding in the tissues, raising some questions concerning the environmental significance of the injection experiments (Whanger et al., 1981). In the present study the route of exposure was of course oral ingestion, and since the two trace elements influenced in terms of their blood concentrations were selenium and cadmium, these two were the elements most likely to have interacted.

The form of selenium in oysters in general, or in O. lutaria in particular, is not known. In man,amino-acid bound forms of selenium (e.g selenomethionine) are well absorbed (about 80%). Data from metabolic studies carried out in the Nutrition Department have indicated that a rise in blood selenium concentration similar to the end-season mean values achieved by Category IV subjects i.e. a rise to above 100 ng/ml can be observed after 4 weeks of selenomethionine supplementation providing 100 μg Se/day (Thomas et al., 1982). Since for Category IV subjects 10 oysters/day would have provided them with about 280 μg selenium, and since they had been ingesting oysters for the preceeding 24 weeks, it seems reasonable to conclude that the selenium from the oysters was reasonably readily available.

None of the studies of Itai-Itai disease patients in Japan included an investigation of selenium, and therefore there is no other data to suggest whether a cadmium-selenium interaction in the present study might have been predicted. Selenium intake would have enhanced by any fish included in diet, as well as by the oysters. Not many subjects ate fish more than once a week.

Thus the data in the present study do not allow any conclusions about possible cadmium-selenium interactions, or whether a protective effect from the selenium in the oysters might have contributed to the lack of effect of the cadmium.

9.3. Cadmium - Iron Interactions

In iron-deficient mice the gastro-intestinal absorption of cadmium was increased (Valberg et al., 1976); conversely high intakes of cadmium appear to markedly depress the absorption of iron from the gut (For et al., 1971). Ascorbic acid, and to a lesser extent copper, could prevent the cadmium-induced anemia (Fox and Fry, 1970; Fox et al., 1971).

In the present study all subjects appeared to have a normal iron status in so far as haemoglobin concentration and packed cell volume measure iron status. Therefore it seemed less likely that the high intake of cadmium from the oysters had any effect on iron absorption. No subject was classified as having a low dietary iron intake; furthermore the small increase in copper intake from the oysters might have provided a small degree of protection against the effect of the cadmium. Although the ascorbic acid status of the subjects was not assessed, many included fruits and vegetables in their diet, so that their ascorbic acid status possibly was no disadvantage in protecting against the effect of the cadmium on iron absorption.

9.4. Cadmium - Calcium Interactions

Exposure to cadmium through oral ingestion has been shown to reduce calcium absorption in animal studies (Richardson and Fox, 1974; Hamilton and Smith, 1977). The alteration in calcium metabolism characteristic in Itai-Itai disease patients is reduced calcification of the skeleton, leading to severe pain in the vertebral column and legs because of bone weakness and to development of fractures from mild trauma (reviewed by Friberg et al., 1974). The exact biochemical and physiological mechanisms responsible for the effects of cadmium on calcium metabolism have not been elucidated, but low dietary intakes of calcium, protein and vitamin D appear to contribute to the etiology.

Both the calcium and the protein intakes of all subjects in the present study were classified either as Very High or as High-Medium; Vitamin D intakes were not assessed. The medical questionnaires contained a question on bone disease; only arthritis or rheumatism (or rheumatoid arthritis) occurred in the survey population. No subjects admitted to any other bone diseases, or to any long or serious illness which could have been related to any known possible effect of a high intake of cadmium.

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APPENDIX A-1

PROTOCOL FOR HANDLING OF PRE-SEASON SAMPLES

1. Labelling

All samples will be labelled with water-proof felt-tip pens with the following:

SURNAME, INITIAL
DATE (day-month)
DESIGNATION (oyster/phos/cheese)

e.g. JONES, B
25,2
oyster

All samples must be carefully checked as they are handed in for correct labelling.

2. Urine

Collection: 2 overnight samples

Handing out: Bottles will be labelled at the time the instructions for collection are explained. Weight of the bottle will be written on the bottom of the bottle and on the lid.

In Bluff: Measure pH. Add NaOH if pH is <5.5 to bring to >5.5. Store refrigerated. (Cool Stores 4°).

Transport: Refrigerated Freight Lines, refrigerated.

In Dunedin: HAS to measure SG, weight and glucose, and to subdivide into aliquots (ca 25 ml) for:

DPH Lab - creatinine
RP - cadmium
HAS - zinc, cadmium, phosphate
?HAS - beta-2-microglobulin

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3. Faeces

Collection: Individual samples for 3 complete days.

Handing out: Empty pottle weights will be written on the bottom of the container and the lid. Each subject to get 4 pottles initially.

In Bluff: Freeze immediately as is. (Cool Stores -25°).

In Dunedin: HAS to weigh samples, freeze dry, grind, pool individual samples to obtain 3-day total samples.
HAS - cadmium, zinc, copper.

4. Blood and Serum

Collection: 1x15 ml heparinised vacutainer - whole blood
1x10 ml plain vacutainer - serum
Vacutainers will be labelled as the blood is taken by JMM.

In Bluff: Whole blood : measure PCV
mix well, refrigerate

Serum: centrifuge, separate serum and refigerate, discard cells.

Transport: Car, refrigerated.

In Dunedin: RP to halve into aliquots
Whole blood - RP: Cd
-MFR: Se, GSHPx (HAS to do Hb)

Serum - RP: Cd
-HAS: ZN, Cu (Fe).

5. Hair

Collection: 1 occipital sample taken (at the time of the blood sample) with stainless steel scissors and trimmed to the 1st inch proximal to the scalp. Storage in small press-top plastic bag.

In Bluff: Store where convenient.

Transport : Via car.

In Dunedin: Send to MD in Auckland via courier pack.

In Auckland: Note hair colour before analysis.

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6. Oysters

Collection: Probably none available pre-seaosn.

Notes on End-of-Season Sampling

Samples collected: as initially, ie urine, faeces, blood, serum, hair,
plus (a) clinical tests: accurate ht, wt, BP

(b) medical interview: medical history
occupational history
dietary history
symptoms (polyuria)

and (c) oysters!

Analysis of samples: as initially, plus
(a) URINE: phosphate,
?creatinine clearance) to be done if initial sampling
uric acid clearance) of oyster men indicates the
phosphate clearance) need for these; to be done on
? amino N,) "oldies" regardless of initial
results.

(b) BLOOD AND SERUM: phosphate, creatinine, urea, calcium,
ferritin.

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APPENDIX A-2

PROTOCOL FOR HANDLING OF END-OF-SEASON SAMPLES

1. Labelling

All containers and samples will be labelled with water-proof felt-tip pens with the following:

SURNAME, INITIALS
DATE (day/month)
DESIGNATION (oyster/control/phos)

e.g.
JONES, B.T.
25/2
CONTROL

All containers must be carefully checked as they are handed in for correct labelling, and noted in the sample record book.

2. Urine

Collection: 2 overnight samples (collected each day).

Handing out: Bottles will be labelled as they are handed out at the time the instructions for collection are explained.

In Bluff: Measure pH and SG. Take 2 aliquots in 10 ml centrifuge tubes
a) for Cd analysis
b) for beta-2-microglobulin
The tubes for b) will have added Na HCO_3.

Storage: Refrigerated (Cool Stores 4°).

Transport: Refrigerated Freight Lines, chilled.
TK to take aliquots for beta-2 analysis to Auckland.

In Dunedin: AC to take aliquots for DPH Lab - creatinine, urea
AC to analyse for Zn.

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3. Faeces

Collection: Individual samples for 3 complete days.

Handing out: Subjects given the option of milkshake pottles or tidy liner plastic bags. Pottles/bags to be put into brown paper bags which are to be labelled when handed out. Each subject to get 2 pottles/bags initially. Samples to be collected each day.

In Bluff: Record in sample record book and freeze as is (Cool Stores -25°).

Transport: Refrigerated Freight Lines, frozen.

In Dunedin: AC to weigh samples, dry, grind and analyse for Zn, Cu, Cd.

4. Blood and Serum

Collection: 1x15ml plain vacutainer - serum
1x15 ml heparinised vacutainer - whole blood
Vacutainers will be labelled as the blood is taken by JMM.

In Bluff: Plain vacutainers: centrifuge, separate serum into 2 vials -
- a) for Zn, Cu, beta-2-microglobulin
- b) for Cd.

Heparinised vacutainers: Measure PCV; separate into aliquots
- a) for Cd
- b) for Se, Hb.

5. Hair

Collection: 1 occipital sample taken (at the time of the blood sample) with stainless steel scissors; mark proximal end; seal in press-top plastic bag. Hair colour and hair treatments to be recorded on medical questionnaire.

In Bluff: Store where convenient.

Transport: TK to take to Auckland.

In Auckland: MD to do duplicate analysis for Cd, Zn, Cu.

6. Dietary History Questionnaire

To be completed for each subject.
Daily records of oyster eating to be collected from 20 subjects.

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7. Medical and Occupational History

To be completed for each subject, plus measurement of height and weight.

8. Oysters

2 dozen to be purchased from each factory; 6 from each 2 dozen to be pooled, dried and analysed for Cd, Zn, Cu, Fe, Mn, Se, Hg, Pb and water content.

APPENDIX B

INSTRUCTION TO SUBJECTS FOR THE COLLECTION OF SAMPLES FOR THE OYSTER SURVEY

Please read this carefully before you start.

NAME

COLLECTIONS:

	Date	Faeces	Urine	Blood	Height and weight
Day 1					
Day 2					
Day 3					

For the three days of this survey you are asked to collect all the faeces passed when your bowels move at any time on the three days, as well as the urine produced overnight on the first 2 of those days. You will be given a large brown paper bag which will contain a large white plastic bottle for the urine, and collection equipment for the faeces. Each day please hand any faeces samples and your urine sample to us.

As well, on one of those three days (   ) a sample of blood and a small sample of hair will be obtained from you, and we will ask you questions about what you eat. We will also measure your height, weight and blood pressure.

How to collect the urine and faeces

1. URINE. Only the urine passed from after the time you go to bed in the evening until when you first get up in the morning is to be collected. If you get up in the night to pass urine put this in the bottle as well as your morning sample. When you get up empty your bladder as completely as possible each time, put the top back tightly onto the bottle when urine is in it. We will give you a separate urine bottle for each day.

2. FAECES. Collect any faeces passed from NOW ON for the next three days directly into the milkshake pottles or the plastic bags (1 for each sample). Please try not to mix urine in with the faeces sample. Flush toilet paper down the toilet. Put the pottle or the plastic bag in the small brown paper bags each day.

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APPENDIX C

PRE-SEASON QUESTIONNAIRE

Name: Date:

Address: Time:

Phone:

Age:

Height: Weight:

Occupation:

Time in present employment:

Previous employment:

Smoking: - Cigarettes: Years of smoking:

Other: Brand smoked:

Oyster consumption: - Last week: Christmas:

Last season (average/week):

Time since last oyster consumed:

Medications: (Especially diuretics)

Hair treatments in the last 2 months:- Perm:

Streak:

Tint:

Anti-dandruff shampoo:

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APPENDIX D

OYSTER STUDY: DIET HISTORY

Name: Date:

1. How often do you eat the following foods:

	Every day	Several times a week	Once a week	A couple of times a month	Once a month or less	Never
Beef or veal	____	____	____	____	____	____
Mutton, hogget or lamb	____	____	____	____	____	____
Pork	____	____	____	____	____	____
Bacon or ham	____	____	____	____	____	____
Sausage	____	____	____	____	____	____
Liver	____	____	____	____	____	____
Kidney (include steak and kidney	____	____	____	____	____	____
Chicken	____	____	____	____	____	____
Eggs	____	____	____	____	____	____
Cream and ice cream	____	____	____	____	____	____
Cheese	____	____	____	____	____	____
Milk	____	____	____	____	____	____
Potato	____	____	____	____	____	____

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	Every day	Several times a week	Once a week	A couple of times a month	Once a month or less	Never
Other root veges (eg carrot, swede)	____	____	____	____	____	____
Green vegetables	____	____	____	____	____	____
Fresh fruit	____	____	____	____	____	____
Cooked fruit (including canned)	____	____	____	____	____	____
Nuts and peanut butter	____	____	____	____	____	____
Dried beans, peas	____	____	____	____	____	____
Rice or pasta	____	____	____	____	____	____
Mushrooms	____	____	____	____	____	____

6-1/2"
9-1/3"

2(a) How many slices of bread and/or toast do you usually eat each day? (Include bread rools and hamburgers: 1 roll or 1 hamburger = 2 slices of bread).

(b) What sort of bread do you usually eat?
1. White
2. Mixed whole grains, wholemeal
3. Brown
4. A mixture of different sorts of bread

3. Do you regularly eat bran or other wholegrain cereals? (eg bread, Bran flakes, All-Bran, wheatgerm as breakfast cereal or in cooking).
No. Yes. (specify)

4. Are you taking any vitamin/mineral pills regularly?
No. Yes. (specify)

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5. As you know we are particularly interested in how much and what type of shellfish (and fish) you eat. How often do you eat (state number):

	4 or more times a week	2-3 times a week	Once a week	2-3 times a month	Once a month	Never
Oysters						
Scallops						
Mussels						
Paua						
Other Shellfish						
Crayfish/shrimp						
Fresh Fish (including fish & chips)						
Canned Fish						
Fish Products (eg fish cakes, fish fingers)						

6. Are you on a special diet (eg vegetarian, weight reducing, low cholesterol)?

No. Yes. (specify)

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MEDICAL QUESTIONNAIRE

Cd-oyster study Date: ____________

1. Name:

2. Age: 3. Height:

4. Weight:

6-1/2"

5. Have you ever been told by a doctor that you have or have had:

9-1/8"

	Yes	No
Chronic bronchitis	___	___
Emphysema	___	___
Asthma	___	___
High blood pressure	___	___
Angina pectoris (chest pain)	___	___
Myocardial infarction, a coronary	___	___
Stroke	___	___
Peptic Ulcer	___	___
Diabetes	___	___
Kidney Stones	___	___
Protein in the urine	___	___
Urinary infection	___	___
Gout	___	___
Any bone disease (which)?____________	___	___
Any congenital or inherited condition (which)?________________	___	___
____________________________		
Any other long or serious illness (which)? ________________________	___	___

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6. Specify further details of disease history:
(when started, symptoms, treatment, outcome):

____

____

____

____

	Yes	No
7. Are you receiving any medication at present?	___	___
Which? (blood pressure pills, diuretics, heart pills, insulin, contraceptive pill....)	___	___

____

6-1/2"

____

How frequently are they taken?

9-1/8"

____

____

	Yes	No
8. Do you, more than once per week, have pain in any part of your body?	___	___
Please describe: (location, frequency, intensity, treatment, outcome)	___	___

____

____

____

____

	Yes	No
9. Are you feeling well at present?	___	___
If no, elaborate: ______________________	___	___

____

____

10. Blood pressure:

11. Smoking habit:

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APPENDIX F

GENERAL INFORMATION

CODE NAME	SEX	AGE yr	HT cm	WT kg	INDEX kg/m^2	OYSTERS doz/wk season average	OYSTER INTAKE CATEGORY	BLOOD PRESSURE mm Hg SYSTOLIC	BLOOD PRESSURE mm Hg DIASTOLIC	SMOKING HABIT cigarettes/day
1.2.CA	F	37	149	52	2.34	1	II	110	68	20
2.2.RA	M	43	175	75	2.45	1	II	120	70	-
3.1.HA	M	40	166	76	2.76	0.1	I	110	75	-
4.2.DB	M	53	179	81	2.53	0.75	II	150	100	-
5.2.MB	F	48	174	72	2.38	0.75	II	130	80	-
6.2.WB	M	23	186	74	2.14	0.75	II	135	70	-
7.1.LB	F	31	*	*	*	0.00	I	*	*	-
8.2.RB	F	47	*	*	*	0.5	II	*	*	20
9.2.PB	F	44	151	52	2.28	1	II	110	70	7
10.2.MB	F	37	152	70	3.03	1	II	110	75	1
11.3.MB	M	36	166	78	2.83	2.5	III	140	85	25
12.4.AB	M	28	*	*	*	7.5	IV	*	*	10
13.3.RB	F	35	144	56	2.70	2.5	III	165	125	20
14.4.TB	M	40	163	85	3.20	10	IV	130	90	20
15.2.VB	M	76	*	*	*	1	II	*	*	-
16.1.MB	F	35	164	75	2.79	0.25	I	100	70	_
17.4.SB	M	31	176	80	2.58	14	IV	130	65	20
18.3.EC	M	59	181	87	2.66	2.5	III	145	80	20
19.1.WC	M	22	169	85	2.98	0.25	I	150	90	-

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APPENDIX F (Continued)

						GENERAL INFORMATION				
CODE NAME	SEX	AGE yr	HT cm	WT kg	INDEX kg/m^2	OYSTERS doz/wk season average	OYSTER INTAKE CATEGORY	BLOOD PRESSURE mm Hg SYSTOLIC	BLOOD PRESSURE mm Hg DIASTOLIC	SMOKING HABIT cigarettes/ day
20.3.CC	M	39	176	78	2.52	2.5	III	125	80	-
21.2.DC	M	32	*	*	*	0.5	II	*	*	-
22.3.DC	M	48	167	87	3.12	2.5	III	140	85	-
23.1.KC	F	32	*	*	*	0.25	I	*	*	-
24.1.MC	M	20	173	62	2.07	0.25	I	130	75	20
25.2.LC	M	51	172	83	2.81	1.5	II	135	95	-
26.4.GC	M	52	183	100	2.99	8	IV	180	100	10
27.3.BD	M	52	172	74	2.50	4.5	III	155	85	-
28.3.MD	M	32	175	79	2.58	3	III	150	110	-
29.4.JE	M	32	178	95	3.00	10	IV	145	100	-
30.1.AE	F	28	*	*	*	0.25	I	*	*	-
31.4.AF	M	29	162	76	2.90	16	IV	115	75	20
32.1.HF	M	50	188	80	2.26	0.25	I	110	70	25
33.2.JG	M	53	166	95	3.45	1.5	II	135	95	-
34.3.PG	M	34	175	86	2.81	4	III	125	65	-
35.3.JG	M	32	165	94	3.45	3	III	135	85	20
36.1.AH	M	61	166	75	2.72	0.25	I	132	65	20
37.3.KH	M	44	176	88	2.84	7	III	140	70	20
38.1.RH	M	24	*	*	*	0.25	I	*	*	-
39.1.NI	M	31	186	80	2.31	0.25	I	170	115	6

EPA-287 (Cin.)
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APPENDIX F (Continued)

CODE NAME	SEX	AGE yr	HT cm	WT kg	INDEX kg/m^2	OYSTERS doz/wk season average	OYSTER INTAKE CATEGORY	BLOOD PRESSURE mm Hg SYSTOLIC	BLOOD PRESSURE mm Hg DIASTOLIC	SMOKING HABIT cigarettes/ day
	GENERAL INFORMATION									
40.1.MJ	F	23	163	70	2.63	0.25	I	120	80	15
41.4.PJ	M	40	153	67	2.52	8	IV	170	100	25
42.2.WJ	M	42	*	*	*	1	II	*	*	25
43.1.PK	M	37	151	80	3.46	0.25	I	150	75	40
44.2.PK	F	36	153	74	3.16	1	II	165	110	20
45.1.RK	M	41	163	70	2.63	0.25	I	165	100	-
46.3.AL	F	25	164	75	2.79	2.5	III	105	65	20
47.4.RL	M	30	*	*	*	7	IV	*	*	-
48.2.PL	M	42	183	98	2.93	0.5	II	130	90	2
49.3.CM	M	23	*	*	*	4	III	*	*	-
50.3.CM	M	46	163	77	2.90	3	III	145	95	25
51.3.SM	F	31	*	*	*	2	III	*	*	-
52.3.PM	M	42	*	*	*	2.5	III	*	*	-
53.3.JM	M	23	168	91	3.22	4	III	150	70	-
54.2.SM	M	23	*	*	*	1	II	*	*	-
55.3.PP	M	23	180	95	2.93	2.5	III	135	80	-
56.4.IP	M	19	*	*	*	10	IV	*	*	10
57.2.MP	M	33	179	87	2.72	0.5	II	130	90	-
58.1.VP	F	30	170	73	2.53	0.25	I	110	70	-

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APPENDIX F (Continued)

CODE NAME	SEX	AGE yr	HT cm	WT kg	INDEX kg/m^2	OYSTERS doz/wk season average	OYSTER INTAKE CATEGORY	BLOOD PRESSURE mm Hg SYSTOLIC	BLOOD PRESSURE mm Hg DIASTOLIC	SMOKING HABIT cigarettes/ day
				GENERAL INFORMATION						
59.2.FP	M	55	157	62	2.52	1.25	II	155	70	-
60.2.GP	M	34	170	70	2.42	1	II	130	80	20
61.3.MP	F	36	153	71	3.03	2	III	130	70	2
62.3.RR	M	33	158	75	3.00	2	III	145	85	-
63.3.PS	M	41	168	83	2.91	3	III	140	105	20
64.2.WS	F	40	160	59	2.28	1.5	II	130	90	20
65.4.TS	M	70	155	75	3.08	7	IV	130	80	-
66.3.JS	F	36	160	72	2.81	3	III	115	70	-
67.1.KS	M	40	*	*	*	0	I	*	*	30
68.3.CT	M	21	160	82	3.20	2.5	III	130	75	20
69.3.TT	M	47	159	73	2.89	3.5	III	120	80	50
70.2.PW	M	24	161	83	3.16	1	II	120	80	-
71.2.AW	M	37	176	75	2.42	1	II	110	70	20
72.2.SW	M	32	164	83	3.09	0.5	II	125	90	-
73.1.AW	M	23	171	64	2.20	0.1	I	130	85	-
74.4.JW	M	25	179	98	3.06	12	IV	140	90	40
75.1.KY	F	18	*	*	*	0.1	I	*	*	15
76.2.TY	M	26	183	79	2.36	1	II	*	*	-

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(4-76)

APPENDIX G-1

PRE-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY I

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
7.1.LB	124.6	39.1	0.68	26.7	483	18.9	101	3.94	150	5.86
16.1.MB	152.0	37.5	3.87	145	486	18.2	62.2	2.33	91.7	3.44
19.1.WC	216.9	44.3	0.56	24.8	321	14.2	52.0	2.30	105	4.65
23.1.KC	886.4	201	0.42	84.4	382	76.7	92.0	18.5	99.7	20.0
30.1.AE	222.5	119	0.95	113.2	547	65.2	58.7	7.00	101	12.1
32.1.HF	269.2	63.3	0.48	30.4	466	29.5	59.2	3.75	170	10.8
36.1.AH	171.5	52.0	0.38	19.8	334	17.4	103	10.61	86.5	4.50
38.1.RH	631.0	103.5	0.49	51.0	217	22.5	33.0	3.42	93.6	9.74
39.1.NI	203.4	56.1	0.86	48.2	555	31.1	69.3	3.89	181	10.2
43.1.PK	161.1	37.4	0.41	15.3	456	17.1	54.8	2.05	58.7	2.20
58.1.VP	591.9	125.0	3.99	499	395	49.4	49.7	6.21	99.8	17.6
67.1.KS	240.3	51.9	0.46	23.9	569	29.5	63.3	3.28	130	6.76
73.1.AW	334.2	96.8	0.98	94.9	513	49.7	54.6	5.28	132	16.4
75.1.KY	110.1	33.1	1.25	41.4	329	10.9	49.1	1.62	105	3.49

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APPENDIX G-2

END-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY I

CODE NAME	FAECAL WEIGHT wet	FAECAL WEIGHT dry	CADMIUM		ZINC		COPPER		MANGANESE	
	g	g	µg/g	µg	µg/g	mg	µg/g	mg	µg/g	mg
3.1.HA	661.1	198.6	0.31	61.6	406	80.7	52.4	10.4	177	35.2
16.1.MB	169.5	29.9	0.93	27.8	501	15.0	74.1	2.22	142	4.24
24.1.MC	113.5	30.5	3.06	93.3	421	12.8	62	1.9	98	3.0
36.1.AH	260.5	50.6	0.34	17.2	259	13.1	54.7	2.77	93.6	4.74
39.1.NI	46.4	16.5	1.02	16.8	440	7.26	78.6	1.30	152.9	2.52
40.1.MJ	100.4	27.4	0.98	26.8	475	13.0	83.6	2.29	173	4.74
43.1.PK	198.2	53.1	1.00	53.1	491	26.1	42.2	2.24	60.8	3.23
58.1.VP	404.2	122.2	0.40	48.9	371	45.3	33.9	4.14	131	16.0
73.1.AW	172.2	54.2	0.87	47.2	554	30.0	60.2	3.26	156	8.46

APPENDIX G-3

PRE-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY II

CODE NAME	FAECAL WEIGHT wet g	dry g	CADMIUM µg/g	µg	ZINC µg/g	mg	COPPER µg/g	mg	MANGANESE µg/g	mg
1.2.CA	88.7	25.3	0.71	18.0	425	10.7	52.0	1.31	95.8	2.42
2.2.RA	139.0	41.6	0.42	17.5	440	18.3	44.0	1.83	135	5.62
4.2.DB	454.0	84.4	1.29	109	405	34.2	68.9	5.81	88.5	7.47
5.2.MB	403.2	94.6	1.32	125	279	26.4	48.1	4.55	73.7	6.97
6.2.WB	311.9	66.6	2.03	135	654	43.6	75.8	10.1	127	8.46
8.2.RB	250.7	57.6	0.70	40.3	277	16.0	40.8	2.35	102	5.88
9.2.PB	116.7	42.2	0.46	19.4	321	13.5	85.8	3.60	153	6.46
10.2.MB	180.5	60.6	0.88	53.3	425	25.8	47.3	2.87	193	11.7
21.2.DC	555.0	139.0	0.41	57.0	382	53.1	46.8	6.51	161	16.9
25.2.LC	350.3	63.3	0.22	13.9	403	25.5	23.8	1.51	61.9	3.92
32.2.JG	372.2	102.1	0.47	48.0	523	53.4	42.8	4.37	112	12.54
44.2.PK	520.6	123.1	0.37	45.5	378	46.5	39.6	4.87	135	16.6
48.2.PL	230.6	56.9	1.85	105.3	483	27.5	46.9	2.67	90.0	5.12
54.2.SM	330.5	75.6	0.47	35.5	302	22.8	25.4	1.92	133	10.0
60.2.GP	244.1	62.9	0.65	40.9	782	49.2	104.9	6.60	153	9.61
64.2.WS	395.3	62.6	2.69	168.4	447	28.0	50.9	3.19	100	6.26
70.2.PW	162.1	39.6	0.77	30.5	440	17.4	60.1	2.38	128	5.07
71.2.AW	98.0	26.5	0.91	24.1	534	14.1	59.0	1.56	119	3.15
72.2.SW	304.9	72.4	0.49	35.5	245	17.7	45.7	3.31	65.5	4.74
76.2.TY	204.7	59.6	7.92	472	638	38.0	97.4	5.80	140	8.36

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APPENDIX G-4

END-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY II

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
1.2.CA	122.4	37.9	1.26	47.9	416	15.8	57.0	2.16	109.5	4.15
2.2.RA	392.0	110.0	1.58	174	398	43.8	64.9	7.14	122.6	13.5
4.2.DB	417.1	83.5	0.51	42.6	448	37.4	61.8	5.16	113.4	9.47
5.2.MB	477.0	98.8	0.43	42.5	425	42.0	64.9	6.42	84.1	8.31
6.2.WB	282.9	74.5	0.61	45.8	514	38.3	51.4	3.83	101.6	7.57
9.2.PB	104.4	29.4	9.58	282	603	17.7	123.1	3.62	85.8	2.52
10.2.MB	149.4	56.6	3.08	175	463	26.2	58.3	3.30	228	12.9
25.2.LC	552.3	98.9	0.42	41.5	477	47.2	46.7	4.62	165	16.3
33.2.JG	354.6	86.1	20.2	1739	816	70.3	78.9	6.79	88.2	7.59
44.2.PK	356.5	78.7	2.64	208	491	38.6	46.1	3.63	138	10.9
48.2.PL	182.5	46.7	9.14	427	641	29.9	91.4	4.27	156	7.29
57.2.MP	189.2	41.9	0.76	31.8	594	24.8	51.2	2.15	142	5.95
59.2.FP	287.9	76.1	2.80	213	475	36.1	74.2	5.65	236	18.0
60.2.GP	375.4	109.4	5.63	616	661	72.3	81.9	8.96	137	15.0
64.2.WS	35.4	11.7	6.21	72.6	689	8.06	90.5	1.06	133	1.55
70.2.PW	23.8	3.9	0.61	2.4	302	1.2	46.3	0.18	103	0.4
71.2.AW	111.5	31.7	0.85	26.9	490	15.5	74.8	2.37	136	4.31
72.2.SW	304.7	77.3	1.03	79.6	232	17.9	37.4	2.89	83.3	6.44
76.2.TY	288.9	62.8	7.88	495	626	39.3	87.7	5.51	143	8.98

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APPENDIX G-5

PRE-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY III

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
11.3.MB	478.1	93.1	0.97	90.3	571	53.2	45.4	4.23	104	9.68
13.3.RB	272.0	58.3	0.62	36.1	494	28.8	53.7	3.13	130	7.58
18.3.EC	235.4	79.7	1.10	87.7	342	27.3	90.0	7.17	99.2	7.91
27.3.BD	342.5	66.5	1.35	89.8	253	16.8	56.3	3.74	88.5	7.83
34.3.PG	516.8	104.8	0.41	43.0	183	19.2	33.7	3.53	65.0	6.81
35.3.JG	148.3	54.5	9.13	498	748	40.8	106	5.78	188	10.23
37.3.KH	133.0	27.3	0.61	16.6	489	13.3	56.4	1.54	41.4	1.13
46.3.AL	299.4	94.0	0.34	32.0	312	29.3	39.2	3.68	100	9.4
49.3.CM	394.6	105.1	0.55	57.8	369	38.8	38.9	4.09	115	12.1
50.3.CM	383.3	65.1	0.89	57.9	373	24.3	54.6	3.55	104	6.77
51.3.SM	158.0	29.9	1.09	32.6	309	9.2	46.5	1.39	102.6	3.07
52.3.PM	74.6	17.4	0.79	13.7	825	14.4	64.0	1.11	97.5	1.70
53.3.JM	205.8	45.9	11.0	505	500	23.0	61.6	2.83	115	5.28
55.3.PP	256.2	72.3	0.37	26.7	409	29.6	31.2	2.26	86.4	6.25
61.3.MP	200.9	36.7	0.61	22.4	533	19.6	55.2	2.03	60.2	2.21
62.3.RR	436.0	79.4	0.45	35.7	420	33.3	42.6	3.38	110	8.73
63.3.PS	172.1	41.4	0.99	41.0	490	20.3	44.6	1.85	103	4.26
66.3.JS	132.2	37.0	0.89	32.9	532	19.7	72.2	2.67	170	6.29
68.3.CT	173.8	28.1	0.55	15.5	442	12.4	60.2	1.69	169	4.75
69.3.TT	354.8	50.6	2.44	124.0	671	34.0	111	5.62	201	10.2

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APPENDIX G-6

END-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY III

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
11.3.MB	351.1	90.3	3.03	121	341	30.8	60.6	5.47	84.7	7.65
13.3.RB	91.2	29.1	23.5	683	565	16.4	69.8	2.03	125.4	3.65
18.3.EC	255.3	46.4	4.07	189	545	25.3	60.1	2.79	112.2	5.21
20.3.CC	129.3	38.7	0.57	22.0	380	14.7	63.0	2.44	181	7.00
22.3.DC	354.5	90.5	2.37	214	652	59.0	79.0	7.15	111	10.0
27.3.BD	235.4	60.5	23.2	1404	625	37.8	94.2	5.70	102.5	6.20
28.3.MD	469.1	107.1	4.53	485	511	55.5	54	5.8	84	8.9
34.3.PG	356.6	65.1	0.92	59.9	380	24.7	38.6	2.52	81.1	5.28
35.3.JG	112.8	36.2	15.0	543	1020	36.9	139	5.03	168	6.08
37.3.KH	193.0	47.7	0.50	23.8	403	19.2	45.8	2.18	53.1	2.53
46.3.AL	140.9	49.0	0.93	45.6	413	20.2	86.0	4.21	125	6.12
50.3.CM	48.5	17.1	8.97	153.4	574	9.81	81.0	1.38	93.6	1.60
53.3.JM	191.0	45.8	6.20	284	374	17.1	53.2	2.44	74.4	3.41
55.3.PP	165.8	44.2	4.26	188	1701	75.2	45.7	2.02	73.6	3.25
61.3.MP	172.4	45.8	34.1	1536	1209	55.4	95.7	4.38	71.3	3.26
62.3.RR	213.3	46.5	5.31	247	584	27.2	64.8	3.01	117	5.44
66.3.JS	127.9	47.9	2.00	95.8	508	24.3	99.1	4.75	163.1	7.81
68.3.CT	91.0	25.3	22.6	57.2	655	16.6	92.0	2.33	150	3.79
69.3.TT	218.6	56.7	8.36	474	564	32.0	122.5	6.94	154	8.74

APPENDIX G-7

PRE-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY IV

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
12.4.AB	166.2	34.1	8.08	275	426	14.53	53.2	1.81	79.6	2.71
14.4.TB	206.8	52.7	1.28	67.5	531	28.0	45.2	2.38	148	7.80
26.4.GC	351.9	84.2	0.42	35.4	535	45.0	64.1	5.40	164	13.8
31.4.AF	128.8	39.4	0.74	29.2	331	13.0	38.5	1.52	98.6	3.88
47.4.RL	379.9	71.2	3.89	277	372	26.5	53.0	3.77	122	8.81
65.4.TS	185.3	25.5	0.35	8.92	486	12.4	50.8	1.29	139	3.54

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APPENDIX G-8

END-SEASON FAECAL DATA FOR OYSTER INTAKE CATEGORY IV

CODE NAME	FAECAL WEIGHT wet g	FAECAL WEIGHT dry g	CADMIUM µg/g	CADMIUM µg	ZINC µg/g	ZINC mg	COPPER µg/g	COPPER mg	MANGANESE µg/g	MANGANESE mg
14.4.TB	90.7	30.5	1.18	36.0	611	18.6	80.0	2.44	343	10.5
17.4.SB	165.5	57.0	16.1	917	706	40.2	61.9	3.53	131.3	7.48
29.4.JE	354.3	75.4	11.36	856	617	46.5	70.3	5.30	83.2	6.27
31.4.AF	64.8	15.3	6.87	105.1	445	6.81	73.1	1.12	90.7	1.39
41.4.PJ	426.2	76.7	4.30	329	564	43.3	58	4.4	107	8.2
65.4.TS	320.0	77.6	14.8	1147	774	60.1	69.1	5.36	102.4	7.95
74.4.JW	224.0	47.1	31.9	1503	714	33.6	71.8	3.38	130	6.15

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APPENDIX H-1

PRE-SEASON AND END-SEASON HAEMOGLOBIN CONCENTRATIONS AND PACKED CELL VOLUMES FOR OYSTER INTAKE CATEGORY I

CODE NAME	PRE-SEASON		END-SEASON	
	HB g/100ml	PCV %	HB g/100ml	PCV %
3.1.HA	*	*	16.0	42
7.1.LB	15.2	44	*	*
16.6.MB	13.5	40	13.0	39
19.1.WC	14.2	46	15.0	48
23.1.KC	14.4	43	*	*
24.1.MC	*	*	15.9	45
30.1.AE	13.0	38	*	*
32.1.HF	14.6	42	15.4	47
36.1.AH	14.7	42	15.5	46
38.1.RH	17.7	47	*	*
39.1.NI	15.3	44	13.0	49
40.1.MJ	*	*	13.0	37
43.1.PK	16.9	47	16.0	43
45.1.RK	16.3	46	14.8	45
58.1.VP	14.1	45	11.0	36
67.1.KS	16.5	49	*	*
73.1.AW	16.9	44	14.8	44
75.1.KY	14.8	42	*	*

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APPENDIX H-2

PRE-SEASON AND END-SEASON HAEMOGLOBIN CONCENTRATIONS AND PACKED CELL VOLUMES FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON		END-SEASON	
	Hb g/100 ml	PCV %	Hb g/100 ml	PCV %
1.2.CA	15.6	44	15.0	42
2.2.RA	17.8	44	16.3	47
4.2.DB	15.8	45	15.6	44
5.2.MB	15.4	44	15.8	46
6.2.WB	16.8	47	15.0	44
8.2.RB	11.8	42	*	*
9.2.PB	11.7	37	11.0	36
10.2.MB	14.6	41	13.9	42
15.2.VB	*	*	*	*
21.2.DC	14.8	*	*	*
25.2.LC	15.5	40	15.4	44
23.2.JG	16.9	46	*	*
44.2.PK	13.4	41	13.8	43
48.2.PL	17.1	44	18.0	47
54.2.SM	15.2	45	*	*
57.2.MP	*	*	14.0	44
59.2.FP	*	*	15.2	43
60.2.GP	14.7	44	14.8	42
64.2.WS	15.7	41	14.3	46
70.2.PW	14.9	43	14.8	45
71.2.AW	14.3	42	14.1	40
72.2.SW	14.8	45	16.3	43
76.2.TY	13.3	43	14.1	41

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APPENDIX H-3

PRE-SEASON AND END-SEASON HAEMOGLOBIN CONCENTRATIONS AND PACKED CELL VOLUMES FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON		END-SEASON	
	Hb g/100 ml	PCV %	HB g/100 mg	PCV %
11.3.MB	15.9	44	16.1	42
13.3.RB	16.1	44	14.8	46
18.3.EC	17.6	44	16.8	44
20.3.CC	*	*	15.2	44
22.3.DC	11.6	38	12.2	37
27.3.BD	13.7	40	13.9	42
28.3.MD	*	*	15.7	39
34.3.PG	15.2	45	15.0	46
35.3.JG	18.0	50	17.0	50
37.3.KH	16.0	45	16.8	47
46.3.AL	11.5	36	14.4	37
49.3.CM	19.2	52	*	*
50.3.CM	16.1	*	16.4	46
51.3.SM	16.6	43	*	*
52.3.PM	16.1	42	*	*
53.3.JM	13.2	43	14.9	40
55.3.PP	14.8	42	16.7	47
61.3.MP	14.0	39	14.3	42
62.3.RR	14.9	45	15.5	45
63.3.PS	13.9	41	14.6	40
66.3.JS	13.1	44	14.2	42
68.3.CT	15.3	42	15.6	44
69.3.TT	15.7	44	16.0	45

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APPENDIX H-4

PRE-SEASON AND END-SEASON HAEMOGLOBIN CONCENTRATIONS AND PACKED CELL VOLUMES FOR OYSTER INTAKE CATEGORY IV

CODE NAME	PRE-SEASON		END-SEASON	
	Hb g/100 ml	PCV %	Hb g/100 ml	PCV %
12.4.AB	17.7	47	*	*
14.4.TB	15.0	37	16.4	50
17.4.SB	*	*	16.1	45
26.4.GC	16.7	46	16.6	47
29.4.JE	*	*	15.8	44
31.4.AF	15.7	47	17.2	46
41.4.PJ	*	*	14.0	41
47.4.RL	15.7	48	*	*
56.4.IP	15.2	45	*	*
65.4.TS	12.5	37	14.5	43
74.4.JW	*	*	15.0	47

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APPENDIX I-1

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY I

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
3.1.HA	*	*	*	*	1.42	0.060	76	68	2.85
7.1.LB	0.99	0.060	*	*	*	*	*	*	*
16.1.MB	1.42	0.044	*	*	1.60	0.050	66	110	1.90
19.1.WC	1.81	0.072	80	75	1.60	0.090	*	*	1.99
23.1.KC	1.18	0.080	*	*	*	*	*	*	*
24.1.MC	*	*	*	*	5.44	0.052	111	93	2.25
30.1.AE	2.16	0.080	*	*	*	*	*	*	*
32.1.HF	6.82	0.068	68	146	6.54	0.074	92	83	2.29
36.1.AH	4.55	0.113	66	96	4.43	0.104	67	113	3.17
38.1.RH	2.07	0.082	*	*	*	*	*	*	*
39.1.NI	4.16	0.076	70	117	4.05	0.083	83	93	2.14
40.1.MJ	*	*	*	*	4.44	0.088	52	93	1.52
43.1.KP	5.83	0.052	57	156	6.16	0.069	68	104	1.56
45.1.RK	1.12	0.051	*	*	1.46	0.058	94	107	2.36
58.1.VP	1.01	0.062	*	*	3.88	0.056	115	150	1.93
67.1.KS	2.13	0.049	*	*	*	*	*	*	*
73.1.AW	0.96	0.045	*	*	0.85	0.040	*	*	2.34
75.1.KY	2.43	0.049	*	*	*	*	*	*	*

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APPENDIX I-2

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
1.2.CA	5.54	0.083	52	119	4.02	0.118	53	73	2.47
2.2.RA	1.16	0.088	68	123	2.03	0.112	84	113	2.00
4.2.DB	1.84	0.046	*	*	2.24	0.062	70	78	1.77
5.2.MB	0.82	0.072	*	*	1.28	0.078	63	117	*
6.2.WB	0.74	0.056	*	*	0.89	0.047	73	153	2.64
8.2.RB	8.67	0.086	52	137	*	*	*	*	*
9.2.PB	4.80	0.075	59	183	4.09	0.071	67	129	2.03
10.2.MB	1.71	0.052	48	148	2.30	0.063	50	197	2.70
15.2.VB	*	0.089	*	*	*	*	*	*	*
21.2.DC	1.33	0.104	*	*	*	*	*	*	*
25.2.LC	1.53	0.057	57	94	1.85	0.055	95	110	3.38
33.2.JC	1.69	0.062	75	112	*	*	*	*	*
44.2.PK	6.93	0.071	68	100	5.12	0.057	81	87	1.61
48.2.PL	0.83	0.082	*	*	1.14	0.081	76	90	2.12
54.2.SM	2.31	0.066	73	113	*	*	*	*	*
57.2.MP	*	*	*	*	4.17	0.063	75	120	1.75
59.2.FP	*	*	*	*	1.39	0.085	71	114	2.37
60.2.GP	3.92	0.071	52	133	3.48	0.067	94	70	3.20
64.2.WS	6.45	0.051	61	104	8.25	0.058	85	83	3.26
70.2.PW	1.49	0.064	55	104	1.39	0.071	42	77	2.05

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APPENDIX I-2 (Continued)

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
71.2.AW	2.11	0.062	61	96	3.66	0.056	67	77	1.64
72.2.SW	1.41	0.072	*	*	1.53	0.072	112	123	1.64
76.2.TY	2.65	0.072	70	119	2.83	0.068	67	107	1.36

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APPENDIX I-3

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
11.3.MB	2.13	0.061	50	127	3.55	0.076	67	101	2.57
13.3.RB	8.82	0.087	73	104	12.37	0.117	67	105	1.82
18.3.EC	3.89	0.091	44	110	7.75	0.093	50	118	2.22
20.3.CC	*	*	*	*	1.24	0.100	68	100	2.13
22.3.DC	1.25	0.059	52	125	1.46	0.084	67	193	1.94
27.3.BD	2.99	0.080	77	129	2.60	0.086	100	107	1.89
28.3.MD	*	*	*	*	2.38	0.089	78	77	2.09
34.3.PG	2.54	0.086	75	115	3.87	0.110	75	90	2.19
35.3.JG	8.60	0.094	82	123	7.57	0.104	106	85	1.83
37.3.KH	2.44	0.058	52	60	2.44	0.057	102	70	2.19
46.3.AL	6.11	0.065	48	238	6.58	0.057	113	220	2.42
49.3.CM	1.96	0.058	*	*	*	*	*	*	*
50.3.CM	2.42	0.084	59	81	4.87	0.093	83	78	1.78
51.3.SM	2.65	0.065	*	*	*	*	*	*	*
52.3.PM	4.09	0.056	68	115	*	*	*	*	*
53.3.JM	1.29	0.074	59	104	1.74	0.075	66	82	1.76
55.3.PP	2.00	0.057	48	75	4.05	0.066	80	63	2.15
61.3.MP	2.20	0.094	59	204	3.52	0.098	75	155	2.37
62.3.RR	0.85	0.069	84	129	3.31	0.091	86	86	1.92

EPA-287 (Cin.)
(4-76)

APPENDIX I-3 (Continued)

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
63.3.PS	6.54	0.077	57	121	8.46	0.090	73	123	2.42
66.3.JS	1.62	0.077	52	108	2.20	0.094	83	95	1.86
68.3.CT	5.44	0.060	75	119	4.34	0.067	74	103	1.74
69.3.TT	4.28	0.066	48	140	3.70	0.104	83	95	2.06

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EPA-287 (Cin.)
(4-76)

APPENDIX I-4

PRE-SEASON AND END-SEASON BLOOD DATA FOR OYSTER INTAKE CATEGORY IV

CODE NAME	PRE-SEASON				END-SEASON				
	WHOLE BLOOD		SERUM		WHOLE BLOOD		SERUM		
	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	Cd ng/ml	Se µg/ml	Zn µg/100ml	Cu µg/100ml	B_2-m µg/l
12.4.AB	0.89	0.066	*	*	*	*	*	*	*
14.4.TB	8.10	0.081	48	100	7.36	0.174	83	93	2.32
17.4.SB	*	*	*	*	9.06	0.074	94	120	3.66
26.4.GC	3.63	0.092	68	125	5.97	0.104	63	130	2.18
29.4.JE	*	*	*	*	2.99	0.107	106	83	1.96
31.4.AF	6.72	0.072	66	140	5.40	0.092	60	67	2.68
41.4.PJ	*	*	*	*	4.34	0.080	61	103	1.67
47.4.RL	2.24	0.078	*	*	*	*	*	*	*
56.4.IP	1.28	0.083	73	151	*	*	*	*	*
65.4.TS	3.16	0.084	45	121	3.70	0.125	72	93	2.89
74.4.JW	*	*	*	*	7.96	0.119	70	73	1.61

EPA-287 (Cin.)
(4-76)

APPENDIX J-1

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY I

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat-inine mmol/l	glucose	protein	SG	pH	creat-inine mmol/l	urea mmol/l	glucose	protein
3.1.HA	*	*	*	*	*	1.017	6.9	6.0	174	-	-
7.1.LB	1.015	6	5.0	-	-	*	*	*	*	*	*
16.1.MB	1.024	5	7.7	-	-	1.028	5.4	7.0	156	-	-
19.1.WC	1.036	5	19.7	-	-	*	*	*	*	*	*
23.1.KC	1.023	7	3.3	-	-	*	*	*	*	*	*
24.1.MC	*	*	*	*	*	1.024	5.8	11.8	212	-	-
30.1.AE	1.032	5	15.8	-	-	*	*	*	*	*	*
32.1.HF	1.031	5	17.3	-	-	1.026	5.4	11.6	480	-	-
36.1.AH	1.022	6	7.2	-	trace	1.021	5.0	6.7	187	-	-
38.1.RH	1.031	5	14.0	-	-	*	*	*	*	*	*
39.1.NI	1.022	6	9.2	-	-	1.027	6.3	28.0	630	-	trace
40.1.MJ	*	*	*	*	*	1.038	6.3	28.0	284	-	trace
43.1.PK	1.022	5	7.6	-	-	1.034	5.4	18.1	650	-	trace
45.1.RK	1.031	5	11.6	-	-	1.034	5.4	14.5	540	-	-
58.1.VP	1.012	7	3.5	-	-	1.020	6.8	6.8	166	-	-
67.1.KS	1.037	5	19.8	-	-	*	*	*	*	*	*
73.1.AW	1.032	5	16.5	-	-	1.034	6.1	15.4	700	-	-
75.1.KY	1.035	5	18.1	-	-	*	*	*	*	*	*

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APPENDIX J-2

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat-inine mmol/l	glucose	protein	SG	pH	creat-inine mmol/l	urea mmol/l	glucose	protein
1.2.CA	1.01	7	7.8	-	-	1.015	6.3	5.2	147	-	-
2.2.RA	1.033	5	18.7	-	-	1.036	5.3	17.9	690	-	-
4.2.DB	1.015	7	5.8	-	-	1.034	6.0	13.6	660	-	trace
5.2.MB	1.024	7	13.2	-	-	1.020	6.6	6.5	185	-	-
6.2.WB	1.028	7	14.2	-	-	1.035	6.6	14.7	690	-	trace
8.2.RB	1.010	6	2.6	-	-	*	*	*	*	*	*
9.2.PB	1.032	6	14.5	-	trace	1.030	6.5	8.5	490	-	-
10.2.MB	1.026	5	12.2	-	-	1.034	5.5	16.6	580	-	trace
15.2.VB	1.015	5	5.0	-	-	*	*	*	*	*	*
21.2.DC	1.028	5	12.8	-	-	*	*	*	*	*	*
25.2.LC	1.028	5	10.5	-	-	1.025	5.7	10.8	204	-	trace
33.2.JG	1.025	5	12.3	-	-	1.026	6.7	7.9	201	-	-
44.2.PK	1.013	5	9.0	-	-	1.025	5.5	10.2	370	-	-
48.2.PL	1.027	5	10.7	-	-	1.024	6.0	8.6	410	-	-
54.2.SM	1.021	5	12.5	-	-	*	*	*	*	*	*
57.2.MP	*	*	*	*	*	1.030	5.7	16.2	485	-	-
59.2.FP	*	*	*	*	*	1.025	6.3	8.3	380	-	trace
60.2.GP	1.012	6	8.6	-	trace	1.027	6.0	10.5	550	-	trace

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APPENDIX J-2 (Continued)

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat-inine mmol/l	glucose	protein	SG	pH	creat-inine mmol/l	urea mmol/l	glucose	protein
64.2.WS	1.012	7	10.0	-	-	1.033	5.6	10.4	510	-	trace
70.2.PW	1.031	6	16.5	-	trace	1.030	6.3	13.9	510	-	-
71.2.AW	1.014	6	5.2	-	-	1.026	5.5	11.5	510	-	-
72.2.SW	1.033		17.7	-	-	1.029	5.8	19.1	300	-	trace
76.2.TY	1.032	6	12.7	-	-	1.036	5.8	18.8	720	-	-

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APPENDIX J-3

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat-inine mmol/l	glucose	protein	SG	pH	creat-inine mmol/l	urea mmol/l	glucose	protein
11.3.MB	1.037	5	19.5	-	trace	1.036	5.4	18.2	640	-	-
13.3.RB	1.022	7	6.5	-	-	1.028	6.1	9.8	540	-	trace
18.3.EC	1.021	7	7.4	-	-	1.020	6.8	13.1	710	-	-
20.3.CC	*	*	*	*	*	1.021	5.8	8.9	184	-	-
22.3.DC	1.033	5	38.4	-	trace	1.026	6.0	11.9	430	-	-
27.3.BD	1.021	5	6.7	-	-	1.034	6.2	11.5	700	-	-
28.3.MD	*	*	*	*	*	1.035	5.6	15.2	660	-	-
34.3.PG	1.032	6	13.8	-	-	1.032	5.5	11.0	610	-	-
35.3.JG	1.031	5	11.0	-	-	1.028	5.7	10.0	530	-	-
37.3.KH	1.016	5	16.7	-	-	1.010	5.5	8.0	177	-	-
46.3.AL	1.019	6	6.8	-	-	1.032	5.7	18.1	550	-	-
49.3.CM	1.035	5	13.6	-	-	*	*	*	*	*	*
50.3.CM	1.025	6	10.8	-	-	1.028	5.6	10.6	540	-	-
51.3.SM	1.022	5	7.4	-	-	*	*	*	*	*	*
52.3.PM	1.023	5	13.3	-	trace	*	*	*	*	*	*
53.3.JM	1.034	5	16.0	-	-	1.039	6.1	16.4	792	-	-
55.3.PP	1.034	6	17.8	-	-	1.030	5.5	15.1	480	-	trace

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APPENDIX J-3 (Continued)

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat- inine mmol/l	glucose	protein	SG	pH	creat- inine mmol/l	urea mmol/l	glucose	protein
61.3.MP	1.029	5	10.3	-	-	1.025	5.1	4.7	450	-	-
62.3.RR	1.031	5	13.4	-	-	1.037	5.5	17.1	880	-	trace
63.3.PS	1.033	5	18.0	-	trace	1.036	5.3	14.7	660	-	-
66.3.JS	1.032	5	18.0	-	trace	1.037	5.5	28.0	710	-	trace
68.3.CT	1.003	6	1.4	-	-	1.027	6.6	7.0	330	-	-
69.3.TT	1.025	7	10.1	-	trace	1.024	6.0	8.2	350	-	-

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APPENDIX J-4

PRE-SEASON AND END-SEASON URINARY DATA (SPECIFIC GRAVITY, pH, CREATININE, UREA, GLUCOSE, PROTEIN) FOR OYSTER INTAKE CATEGORY IV

CODE NAME	PRE-SEASON					END-SEASON					
				Combur tests						Combur tests	
	SG	pH	creat-inine mmol/l	glucose	protein	SG	pH	creat-inine mmol/l	urea mmol/l	glucose	protein
12.4.AB	1.033	5	29.2	-	-	*	*	*	*	*	*
14.4.TB	1.016	5	6.1	-	-	1.025	5.4	12.8	480	-	-
17.4.SB	*	*	*	*	*	1.027	6.1	9.0	470	-	trace
26.4.GC	1.016	*	5.7	-	-	*	*	*	*	*	*
29.4.JE	*	*	*	*	*	1.032	6.6	5.8	187	-	trace
31.4.AF	1.036	5	27.0	-	-	1.039	6.2	14.2	660	-	trace
41.4.PJ	*	*	*	*	*	1.026	4.7	12.8	173	-	-
47.4.RL	1.028	6	12.4	-	-	*	*	*	*	*	*
56.4.IP	1.023	7	14.6	-	-	*	*	*	*	*	*
65.4.TS	1.013	7	3.3	-	trace	1.015	6.7	2.7	123	-	-
74.4.JW	*	*	*	*	*	1.030	5.1	14.0	450	-	-

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APPENDIX K-1

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN) FOR OYSTER INTAKE CATEGORY I

CODE NAME	PRE-SEASON					END-SEASON				
	CADMIUM		ZINC		B_2-m	CADMIUM		ZINC		B_2-m
	ng/ml	µmol/mol creat.	µg/ml	mmol/mol creat.	µg/l	ng/ml	µmol/mol creat.	µg/ml	mmol/mol creat.	µg/l
3.1.HA	*	*	*	*	*	1.51	2.31	0.17	0.43	89
7.1.LB	1.59	2.92	0.24	0.74	*	*	*	*	*	*
16.1.MB	2.04	2.43	0.51	1.02	*	2.53	3.31	0.60	1.32	51
19.1.WC	2.32	1.08	0.80	0.62	85	*	*	*	*	*
23.1.KC	3.19	8.87	0.09	0.42	*	*	*	*	*	*
24.1.MC	*	*	*	*	*	0.32	0.25	0.63	0.82	69
30.1.AE	*	*	*	*	*	*	*	*	*	*
32.1.HF	2.51	1.33	1.22	1.08	64	2.55	2.02	0.64	0.85	52
36.1.AH	1.31	1.67	0.29	0.62	154	2.53	3.46	0.25	0.57	136
38.1.RH	2.02	1.32	0.70	0.77	*	*	*	*	*	*
39.1.NI	1.83	1.82	0.71	1.19	101	1.76	0.58	0.63	0.35	42
40.1.MJ	*	*	*	*	*	1.29	0.42	0.48	0.26	143
43.1.PK	0.6	0.82	0.30	0.61	106	1.44	0.73	0.69	0.59	31
45.1.RK	2.24	1.77	0.70	1.01	*	1.11	0.70	0.86	0.91	129
58.1.VP	0.96	2.52	0.08	0.35	*	4.61	6.22	0.18	0.41	45
67.1.KS	2.36	1.09	1.40	1.09	*	*	*	*	*	*
73.1.AW	1.76	0.98	0.69	0.64	*	1.51	0.90	0.85	0.85	60
75.1.KY	3.76	1.90	0.38	0.32	*	*	*	*	*	*

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APPENDIX K-2

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN) FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON					END-SEASON				
	CADMIUM		ZINC			CADMIUM		ZINC		
	ng/ml	µmol/mol creat.	µg/ml	mmol/mol creat.	B_2-m µg/l	ng/ml	µmol/mol creat.	µg/ml	mmol/mol creat.	B_2-m µg/l
1.2.CA	0.69	0.81	0.08	0.16	43	0.82	1.45	0.07	0.21	17
2.2.RA	2.26	1.11	0.68	0.56	*	2.91	1.49	0.66	0.57	34
4.2.DB	0.99	1.56	0.13	0.34	*	0.54	0.36	0.32	0.36	261
5.2.MB	2.08	1.44	0.39	0.45	*	3.62	5.11	0.12	0.28	89
6.2.WB	2.79	1.80	0.48	0.52	*	1.79	1.12	0.44	0.46	112
8.2.RB	1.60	5.64	0.10	0.59	52	*	*	*	*	*
9.2.PB	4.11	2.60	0.32	0.34	*	1.78	1.92	0.23	0.42	88
10.2.MB	1.99	1.50	0.67	0.84	60	2.16	1.19	1.19	1.10	42
15.2.VB	1.85	3.39	0.34	1.05	*	*	*	*	*	*
21.2.LC	1.41	1.01	0.38	0.46	*	*	*	*	*	*
25.2.LC	1.22	1.06	0.37	0.54	1.5	1.78	2.53	0.46	0.65	100
33.2.JG	1.63	1.21	0.42	0.52	*	1.88	2.18	0.60	1.17	94
44.2.PK	1.22	1.24	0.27	0.46	49	0.70	0.63	0.72	1.08	45
48.2.PL	3.19	2.73	0.38	0.55	*	3.89	4.15	0.27	0.48	42
54.2.SM	0.70	0.51	0.91	1.12	70	*	*	*	*	*
57.2.MP	*	*	*	*	*	3.25	1.84	0.41	0.39	58
59.2.FP	*	*	*	*	*	1.08	1.19	0.46	0.85	26
60.2.GP	0.23	0.24	0.71	1.27	57	2.53	2.21	0.77	1.13	73

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APPENDIX K-2 (Continued)

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN) FOR OYSTER INTAKE CATEGORY II

CODE NAME	PRE-SEASON CADMIUM ng/ml	PRE-SEASON CADMIUM $\frac{\mu mol}{mol}$ creat.	PRE-SEASON ZINC µg/ml	PRE-SEASON ZINC $\frac{mmol}{mol}$ creat.	PRE-SEASON B_2-m µg/l	END-SEASON CADMIUM ng/ml	END-SEASON CADMIUM $\frac{\mu mol}{mol}$ creat.	END-SEASON ZINC µg/ml	END-SEASON ZINC $\frac{mmol}{mol}$ creat.	END-SEASON B_2-m µg/l
64.2.WS	2.58	2.37	0.36	0.55	130	3.98	3.51	0.68	1.01	155
70.2.PW	1.08	0.60	0.57	0.53	89	1.14	0.75	0.63	0.70	94
71.2.AW	0.18	0.32	0.14	0.41	*	0.50	0.40	0.32	0.43	42
72.2.SW	3.21	1.60	1.14	0.99	*	2.33	1.12	1.27	1.02	105
76.2.TY	0.95	0.69	0.40	0.48	224	1.15	0.56	0.96	0.78	58

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APPENDIX K-3

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN) FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON CADMIUM ng/ml	PRE-SEASON CADMIUM µmol/mol creat.	PRE-SEASON ZINC µg/ml	PRE-SEASON ZINC mmol/mol creat.	PRE-SEASON B_2-m µg/l	END-SEASON CADMIUM ng/ml	END-SEASON CADMIUM µmol/mol creat.	END-SEASON ZINC µg/ml	END-SEASON ZINC mmol/mol creat.	END-SEASON B_2-m µg/l
11.3.MB	4.97	2.34	0.70	0.55	*	5.33	2.69	0.72	0.61	38
13.3.RB	6.53	9.22	0.50	1.18	110	8.75	8.19	0.57	0.89	68
18.3.EC	2.85	3.53	0.11	0.23	54	4.45	3.12	0.05	0.06	73
20.3.CC	*	*	*	*	*	1.05	1.08	0.27	0.47	102
22.3.DC	5.72	1.37	1.05	0.42	30	5.87	4.52	0.12	0.15	62
27.3.BD	1.33	1.82	0.40	0.92	*	1.20	0.96	0.49	0.65	168
28.3.MD	*	* *	*	*	*	1.84	1.11	0.60	0.61	54
35 3.JG	1.47	1.23	0.56	0.78	125	1.51	1.38	0.60	0.92	93
37.3.KH	1.67	0.92	1.07	0.98	*	1.08	1.24	0.29	0.56	10
46.3.AL	1.28	1.73	0.51	1.15	98	1.67	0.85	0.62	0.53	62
49.3.CM	1.72	1.16	0.79	0.89	*	*	*	*	*	*
50.3.CM	2.89	2.45	0.95	1.35	96	1.48	1.28	0.78	1.13	34
51.3.SM	2.26	2.80	0.32	0.66	*	*	*	*	*	*
52.3.PM	5.15	3.55	0.95	1.10	65	*	*	*	*	*
53.3.JM	1.22	0.70	0.87	0.84	62	1.94	1.08	0.46	0.43	49
55.3.PP	1.83	0.94	0.41	0.35	136	4.90	2.98	0.59	0.60	64
61.3.MP	1.06	0.94	0.43	0.64	76	2.53	4.94	0.17	0.56	31
62.3.RR	1.47	1.01	0.90	1.03	45	0.95	0.51	0.90	0.81	95

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APPENDIX K-3 (Continued)

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN)
FOR OYSTER INTAKE CATEGORY III

CODE NAME	PRE-SEASON					END-SEASON				
	CADMIUM		ZINC			CADMIUM		ZINC		
	ng/ml	$\frac{\mu mol}{mol}$ creat.	µg/ml	$\frac{mmol}{mol}$ creat.	B_2-m µg/l	ng/ml	$\frac{\mu mol}{mol}$ creat.	µg/ml	$\frac{mmol}{mol}$ creat.	B_2-m µg/l
63.3.PS	4.38	2.23	1.21	1.03	*	5.08	3.17	0.87	0.91	33
66.3.JS	1.06	0.54	0.35	0.30	98	1.52	0.50	0.53	0.29	66
68.3.CT	<.01	0.65	0.06	0.66	14	1.82	2.38	0.26	0.57	71
69.3.TT	1.45	1.32	0.16	0.24	*	4.16	4.65	0.49	0.92	40
34.3.PG	3.20	2.13	0.71	0.79	216	0.88	0.73	0.82	1.15	93

APPENDIX K-4

PRE-SEASON AND END-SEASON URINARY DATA (CADMIUM, ZINC AND B_2-MICROGLOBULIN) FOR OYSTER INTAKE CATEGORY IV

CODE NAME	PRE-SEASON CADMIUM ng/ml	PRE-SEASON CADMIUM µmol/mol creat.	PRE-SEASON ZINC µg/ml	PRE-SEASON ZINC mmol/mol creat.	PRE-SEASON B_2-m µg/l	END-SEASON CADMIUM ng/ml	END-SEASON CADMIUM µmol/mol creat.	END-SEASON ZINC µg/ml	END-SEASON ZINC mmol/mol creat.	END-SEASON B_2-m µg/l
12.4.AB	1.55	0.49	1.19	0.63	*	*	*	*	*	*
14.4.TB	0.88	1.32	0.30	0.76	106	0.78	0.56	1.02	1.22	85
17.4.SB	*	**	*	*	*	3.21	3.27	0.28	0.48	73
26.4.GC	0.88	1.42	0.10	0.27	50	*	*	*	*	*
29.4.JE	*	**	*	*	*	1.28	2.02	0.69	1.83	75
31.4.AF	5.31	1.80	1.03	0.59	428	2.06	1.32	0.28	0.30	180
41.4.PJ	*	*	*	*	*	2.36	1.69	0.90	1.08	15
47.4.RL	2.80	2.07	0.92	1.02	*	*	*	*	*	*
56.4.IP	1.76	1.10	1.01	1.06	122	*	*	*	*	*
65.4.TS	0.68	1.89	0.08	0.37	42	0.43	1.46	0.03	1.71	27
74.4.JW	*	*	*	*	*	2.08	1.36	0.78	0.86	32

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APPENDIX L-1

PRE-SEASON AND END-SEASON HAIR DATA FOR OYSTER INTAKE CATETORY I

CODE NAME	PRE-SEASON Cd µg/g	PRE-SEASON Zn µg/g	PRE-SEASON Cu µg/g	END-SEASON Cd µg/g	END-SEASON Zn µg/g	END-SEASON Cu µg/g
3.1.HA	*	*	*	*	185	20.6
7.1.LB	0.37	183	45.8	*	*	*
16.1.MB	0.67	248	4.5	0.06	175	19.4
19.1.WC	0.12	162	10.8	0.28	167	8.7
23.1.KC	0.23	230	15.3	*	*	*
24.1.MC	*	*	*	0.30	209	16.2
30.1.AE	*	177	18.6	*	*	*
32.1.HF	0.24	146	18.3	0.17	159	16.1
36.1.AH	0.49	188	29.7	0.25	134	19.3
38.1.RH	0.11	218	17.9	*	*	*
39.1.NI	0.52	149	11.5	0.12	168	18.7
40.1.MJ	*	*	*	0.10	169	40.6
43.1.PK	0.32	215	17.7	0.15	178	14.4
45.1.RK	0.81	136	20.3	0.36	156	29.7
58.1.VP	*	276	14.1	*	*	*
67.1.KS	0.25	202	8.4	*	*	*
73.1.AW	0.08	228	16.9	0.04	161	17.1
75.1.KY	0.31	164	16.4	*	*	*

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APPENDIX L-2

PRE-SEASON AND END-SEASON HAIR DATA FOR OYSTER INTAKE CATETORY II

CODE NAME	PRE-SEASON Cd µg/g	 Zn µg/g	 Cu µg/g	END-SEASON Cd µg/g	 Zn µg/g	 Cu µg/g
1.2.CA	0.46	223	17.3	0.07	163	15.1
2.2.RA	0.95	198	11.0	0.15	197	18.9
4.2.DB	*	216	57.0	0.14	188	11.9
5.2.MB	0.62	187	15.6	0.05	188	19.5
6.2.WB	0.33	165	13.0	0.18	147	14.8
8.2.RB	0.96	189	18.7	*	*	*
9.2.PB	0.34	123	22.7	0.21	147	32.5
10.2.MB	0.17	240	13.8	0.06	167	7.5
15.2.VB	0.26	149	10.2	*	*	*
21.2.DC	0.19	200	12.1	*	*	*
25.2.LC	0.56	217	23.6	0.36	191	22.7
33.2.JG	*	*	*	*	225	12.8
44.2.PK	0.15	211	14.1	0.11	171	12.7
48.2.PL	0.21	188	10.4	*	161	22.6
54.2.SM	*	234	26.5	*	*	*
57.2.MP	*	*	*	0.20	151	8.6
59.2.FP	*	*	*	0.18	148	8.3
60.2.GP	0.49	264	17.6	*	*	*
64.2.WS	0.41	168	12.1	0.07	128	6.7
70.2.PW	0.57	196	17.8	0.13	206	19.0
71.2.AW	0.28	163	22.6	0.56	151	25.1
72.2.SW	*	*	*	0.18	226	17.1
76.2.TY	0.59	179	12.2	0.22	154	9.9

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APPENDIX L-3

PRE-SEASON AND END-SEASON HAIR DATA FOR OYSTER INTAKE CATETORY III

CODE NAME	PRE-SEASON Cd µg/g	PRE-SEASON Zn µg/g	PRE-SEASON Cu µg/g	END-SEASON Cd µg/g	END-SEASON Zn µg/g	END-SEASON Cu µg/g
11.3.MB	0.79	252	18.7	0.38	186	7.0
13.3.RB	0.11	185	16.8	0.07	140	11.8
18.3.EC	0.78	221	20.4	0.12	202	18.3
20.3.CC	*	*	*	*	*	*
22.3.DC	0.45	156	17.9	*	*	*
27.3.BD	0.17	155	9.7	*	*	*
28.3.MD	*	*	*	0.38	173	29.7
34.3.PG	0.36	157	14.2	0.36	180	27.3
35.3.JG	0.36	168	14.0	0.12	148	9.5
37.3.KH	0.55	196	44.7	*	193	19.7
46.3.AL	0.15	216	11.1	0.10	164	44.0
49.3.CM	0.87	169	20.1	*	*	*
50.3.CM	*	250	16.2	*	*	*
51.3.SM	*	204	12.4	*	*	*
52.3.PM	0.12	139	18.6	*	*	*
53.3.JM	0.69	221	11.4	0.51	181	13.8
55.3.PP	0.33	187	22.5	0.27	161	15.0
61.3.MP	*	253	46.1	*	*	*
62.3.RR	0.57	153	16.8	0.25	150	11.2
63.3.PS	*	*	*	0.48	172	18.6
66.3.JS	0.37	114	20.4	0.29	171	47.5
68.3.CT	0.96	174	17.4	0.63	196	22.6
69.3.TT	0.41	219	13.2	0.11	176	11.9

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APPENDIX L-4

PRE-SEASON AND END-SEASON HAIR DATA FOR OYSTER INTAKE CATETORY IV

CODE NAME	PRE-SEASON Cd µg/g	 Zn µg/g	 Cu µg/g	END-SEASON Cd µg/g	 Zn µg/g	 Cu µg/g
12.4.AB	*	350	16.7	*	*	*
14.4.TB	0.69	174	8.7	0.14	158	8.5
17.4.SB	*	*	*	0.40	133	15.2
26.4.GC	0.55	195	15.5	0.41	139	19.8
29.4.JE	*	*	*	0.63	167	33.5
31.4.AF	0.41	157	8.5	0.19	160	11.2
41.4.PJ	*	*	*	0.90	175	20.5
47.4.RL	0.14	140	11.7	*	*	*
56.4.IP	0.20	175	13.1	*	*	*
65.4.TS	0.25	269	*	0.16	179	15.3
74.4.JW	*	*	*	0.09	127	7.3

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